Whitman (C. O.)

A CONTRIBUTION

TO THE HISTORY OF THE

GERM-LAYERS IN CLEPSINE.

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REPRINTED FROM THE JOURNAL OF MORPHOLOGY,
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CONTENTS.

INTRODUCTORY.

THE origin and fate of the germ-layers in Clepsine have been the subject of investigation and critical discussion by a number of recent writers, who have reached conclusions so widely at variance that one might still say with Balfour, — "Our knowledge of the development of the Discophora is in a very unsatisfactory state." The origin of the mesenteron is very difficult to trace, and ignorance of its history has led to the greatest con-

fusion in the identification of the germ-layers, and to most contradictory interpretations of the strata composing the germ-bands. The association of neuroblastic with mesoblastic elements in the germ-bands, as maintained in my first paper on this subject, proved a serious stumbling-block to Balfour.

The germ-bands in a closely related group of annelids, the Oligochæta, were held to be purely mesoblastic, and my suggestion that they contained a neural stratum appeared to stand in plain contradiction with well-established views as to the origin of the nervous system. Moreover, Kowalevsky, whose brilliant success in extending the germ-layer theory to the invertebrates had rendered his authority preëminent, had stated, as a fact settled by his own observations, that the nervous system of Clepsine, like that of Lumbricus and other oligochætous annelids, was derived from the "upper layer," *i.e.*, the epidermal layer. This statement, although based upon an evidently hasty and unreliable examination of a few poorly preserved eggs of Clepsine, was corroborated by more extended studies on Rhynchelmis (Euaxes) and Lumbricus, and later, by the researches of Hatschek, Kleinenberg, and others. The more trustworthy statements of Metschnikoff, published in the same year with Kowalevsky's "Embryological Studies on Worms and Arthropods," escaped the attention of Balfour, and thus the testimony of numerous excellent observers as well as theoretical considerations appeared to stand in the way of accepting my conclusions. The contradictory results since reached by Bergh and Nusbaum have only made it still more desirable to reëxamine the subject with greater care and thoroughness.

Bergh's important researches on the development of the Gnathobdellidæ have led him to dispute the concurrent testimony of previous investigators on the origin of the epidermal layer in Clepsine; and thus we are left in a state of uncertainty regarding the origin and limitations of the germ-layers, not very far removed from that in which Hoffmann found himself, when, at the end of his second memoir on this subject, he frankly confessed his inability to distinguish "Keimblätter" in the Hirudinea.

Respecting the histological differentiation of the germ-band strata, there is still less unanimity of opinion. Even the latest writers, Bergh and Nusbaum, have failed to agree on the

derivation of the ventral nerve-cord, and my studies have led me to results which contradict the conclusions of both these authors. Although the origin and development of the nephridia and the sexual organs have received special attention in recent papers, these questions are still very far from being definitely settled. The precise origin of the nephridia forms just now a question of considerable theoretical importance, — an importance which will be found to be very much increased by the facts to be presented in this paper, and by the parallel results reached by Wilson in his study of Lumbricus. We have long been prepared to believe in the homology of the germ-bands of the Hirudinea and the Oligochæta; but such a complete parallel, both in the mode of origin and in the histological development, as is shown in these two papers, will certainly be a surprise to most embryologists. The observations have been made, independently, on representatives of two different groups of annelids, and they confirm each other in a manner too positive to leave any room for a reasonable doubt of their essential accuracy. Allowing that they are correct, it will be seen that they furnish what we have long stood in need of, — a satisfactory basis for the comparative study of the germ-layers in annelids, — and that they give us one more clue to the ancestral history of the vertebrates.

For the observations recorded in this paper, I had only a single batch of eggs of Clepsine parasitica (?) Say, and a few eggs of C. complanata obtained at Naples. This scanty material did not enable me to carry the investigation beyond the stage in which the concrescence of the germ-bands is nearly completed. I am now able, however, to account for the origin of all the germ-layers, and to give the earlier history of the germ-bands. I have been able to add something to our knowledge of the development of the head, and I hope soon to obtain material for a thorough study of this very important part of the subject. The methods employed have been described in the "American Naturalist," Nov., 1885, pp. 1134–1135; and a preliminary notice of the results obtained was published in the "Zoologischer Anzeiger," No. 218, 1886.

I. CLEAVAGE, AND THE EARLY ESTABLISHMENT OF BILATERALLY SYMMETRICAL RELATIONS.

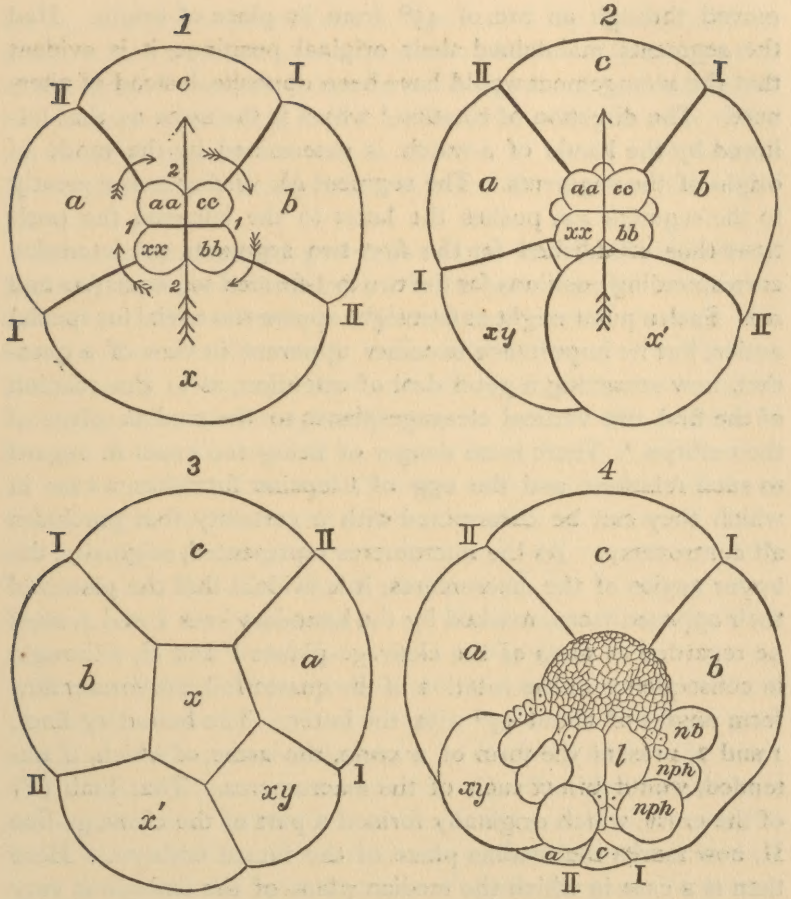
An accurate acquaintance with the principal events of cleavage is indispensable to a clear understanding of the derivation of the germ-layers; for these layers, and even the more important organs arising from them, can be traced directly to special blastomeres.

First Period, ending with the Eight-cell Stage.—A sketch of the cleavage, based upon results published in an earlier paper (No. 1, pp. 49–58, 75–76) is here introduced for the sake of clearness in the discussion and descriptions which are to follow.

The first two cleavage-planes are vertical, dividing the egg into four macromeres, three of which are relatively small, but nearly equal. The fourth, larger segment, which contains the remnants of the polar rings characteristic of this egg, occupies a *posterior* position with reference to the future embryo, while the *anterior* and the two *lateral* positions are held by the three smaller macromeres. The next step consists in the formation of four ectoblastic micromeres, which eventually present the figure of a quarterfoil at the animal pole. The posterior macromere (Diag. 1, *x*) first buds off the ectoblast *xx*, then the right macromere, *b*, gives rise to the second ectoblast, *bb*, and immediately afterward the third and fourth ectoblasts, *aa*, and *cc*, are produced simultaneously by the left macromere, *a*, and the anterior macromere *c*. We arrive thus at the eight-cell stage so common among worms and molluscs, represented by four macromeres surmounted by four micromeres that lie in cruciform order in the boundary-lines of the mother-cells.

The complete orientation of this stage embraces some relations of position yet to be noticed. The alternation of the micromeres with the macromeres is an arrangement brought about by a rotation of the whole quarterfoil, each segment having

(6.) Embryology of Clepsine. *Quart Journ. Mic. Sci.*, XVIII., 1878.



Semi-diagrammatic surface views of the egg of Clepsine in different stages of Cleavage.

Diag. 1. — The eight-cell stage, showing the relation of the embryonic axis to the first two cleavage-planes. The arrow, 2–2, shows the median plane of the embryo, and the four small arrows indicate the direction in which the four micromeres have rotated on the axis of the egg.

Diag. 2. — The posterior macromere (Diag. 1, *x*) has just divided into the neuro-nephroblast *x'*, and the two mesoblasts *x* and *xy*.

Diag. 3. — Same stage seen from the lower pole.

Diag. 4. — Stage in which all the teloblasts and the blastodisc have been formed.

I–I and 1–1. — First cleavage-plane; II–II and 2–2, second cleavage plane; *a*, left macromere; *c*, median and anterior macromere; *b*, right macromere; *x*, posterior macromere; *aa*, *cc*, *bb*, *xx*, micromeres derived from *a*, *c*, *b*, and *x'*, neuro-nephroblast; *xy*, left mesoblast; *x* (Diag. 3.), right mesoblast; *nb*, neuroblast; *nph*, nephroblast; *l*, lateral (median in this stage) teloblast.

moved through an arc of 45° from its place of origin. Had the segments maintained their original positions, it is evident that the arrangement would have been opposite, instead of alternate. The direction of rotation,[1] which is the same as that followed by the hands of a watch, is determined by the mode of origin of the segments. The segment *bb*, arising subsequently to the segment *xx*, pushes the latter to the left, and the positions thus established for the first two segments predetermine corresponding positions for the two last-formed segments (*aa* and *cc*). Such a point might at first sight appear too trivial for special notice, but its importance becomes apparent in view of a question, now attracting a good deal of attention, as to the relation of the first two vertical cleavage-planes to the median plane of the embryo. There is no danger of being too exact in regard to such relations, and the egg of Clepsine furnishes a case in which they can be determined with a certainty that precludes all controversy. As the micromeres represented, originally, the upper angles of the macromeres, it is evident that the planes of their apposed faces, marked by the boundary lines 1 and 2, must be regarded as parts of the cleavage-planes I and II, although, in consequence of the rotation of the quarterfoil, the former now form angles of about 45° with the latter. The boundary lines, 1 and 2, present the form of a cross, the arms of which, if extended, would bisect each of the macromeres. That limb (2) of the cross, which originally formed a part of the cleavage-line II, now marks the median plane of the future embryo. Here then is a case in which the median plane of the embryo is very clearly defined, and in which it coincides with neither of the first two cleavage-planes, but forms an angle of 45° with each of them.

The cleavages resulting in the eight-cell stage are comparable with the first two meridional cleavages and the first equatorial or horizontal cleavage in the eggs of amphibia and some fishes.

The further history of the quarterfoil of micromeres has not been worked out with sufficient thoroughness to say positively and precisely what part they play in forming the embryo; but it seems quite certain that they contribute to the formation of

[1] A similar rotation has been described by Hatschek (No. 35, p. 6) in Eupomatus.

the epidermal stratum of the ectoderm, and it is possible that they are directly employed in the formation of more important parts of the head. Smaller micromeres are gradually added round the primary quarterfoil, in the proliferation of which each of the macromeres appears to share. A disc of small cells is thus formed in which it is impossible to trace the genetic history of individual elements.

Second Period, ending with the Formation of Proliferating Blastomeres. — All regularity of cleavage ends with the eight-cell stage. Henceforth several distinct forms of cleavage will be carried on simultaneously, each restricted to special areas or blastomeres. Although there is scarcely anything in the external appearance of the eight-cell stage to indicate the relation of its parts to the future embryo, yet we know by what follows that an immense work has already been accomplished. All those fundamental conditions and relations implied in the terms anterior and posterior, right and left, dorsal and ventral, are now definitely established. The ground-plan of the future structure is there, and the segregation and distribution of the building material have advanced far toward completion.

The second period concludes the finishing strokes of cleavage, carrying us from the eight-cell stage to that in which the proliferation of the germ-bands begins. The prospective character of the work becomes more and more manifest, and architectural forecasts begin to reveal themselves. It is a period of preparation in which everything is ordered and appointed for the *formative* work with which the third or embryonic period begins. Proliferating blastomeres are created, grouped, and stationed according to the special kinds of work which they, as the artisans of the third period, are destined to accomplish. There are exactly *thirteen* of these blastomeres, symmetrically arranged in *three* primary groups. One group, consisting of *three entoblasts*, is represented by the macromeres, *a*, *b*, and *c*, or rather, will be represented by them at the end of this period, after they have ceased to contribute to the ectoblastic disc. The second and third groups, consisting respectively of *two mesoblasts* and *eight ectoblasts*, are yet to be developed from the large posterior macromere, *x*. The macromeres *a*, *b*, and *c* take no further part in the cleavage, if we except the budding off of ectoblastic micromeres at the animal pole, which does not sensibly diminish their size or alter

their general appearance. Throughout the embryonic period, until long after hatching, these huge segments preserve their individuality, only undergoing such slight changes in form and position as are induced by the development of the germ-bands and the epibolic expansion of the ectoderm. They contain most of the food-yolk, which is utilized in the long post-embryonic pseudo-larval period, and the elements which are to form the mesenteron.

The posterior macromere, x, on the other hand, undergoes sucessive cleavages, resulting in the production of *ten blastomeres*, or *teloblasts*, arranged in two bilaterally symmetrical groups, at the posterior edge of the blastodisc (Diag. 4).

The first cleavage-plane runs obliquely, beginning a little to the left of the upper angle and taking a slanting direction towards the right side, thus cutting off (x, Diag. 2) about one-third of the original macromere. Then follows a second cleavage, at right angles to the first, cutting the larger segment into nearly equal parts (x and xy, Diag. 3). The posterior macromere is now represented by three sub-equal segments: one of these, x', which may be called the *neuro-nephroblast* ("primary neuroblast," in my first paper), lies at the posterior edge of the blastodisc, more on the right than the left side. The second and third, representing the *mesoblasts*, are also asymmetrically placed, one (x) occupying a central position at the lower pole of the egg (Diag. 3), the other ($x\ y$) lying behind it, and to the left of the neuro-nephroblast (x'). Neither in the general appearance nor in the relative positions of the two mesoblasts is there anything indicative of their homotypical character. They appear to be vitelline spheres of the same nature as the three macromeres, a, b, and c, and have always been so regarded by earlier observers. In the course of this period a shifting of position among the cleavage-spheres takes place, which brings the mesoblasts into harmony with the bilateral symmetry of the egg. The three macromeres lengthen backward, slowly flowing over the mesoblasts and more or less completely enveloping them, so that one or both of them may entirely disappear from view, or, at least, become so obscure in outline as not to be easily recognized. In its backward elongation the anterior macromere c takes up a median ventral position between a and b, and usually carries the mesoblast x towards the hind end at the same time that it incloses it.

The right mesoblast x thus becomes imbedded mainly in c, but usually lies partly in b. Meanwhile, the mesoblast, xy, takes up an opposite position in the left macromere, a. I have never seen a single case in which bilateral symmetry was complete with respect to the mesoblasts; but the final position is always such that each proliferates exclusively for the germ-band of its own side. Their relative positions in transverse section are shown in Fig. 2, Pl. IV. In Fig. 3, representing a sagittal section of *C. complanata*, the right mesoblast, x, is placed much farther forward than is usually the case in *C. marginata*.

By successive vertical cleavages the neuro-nephroblast is split up into eight octoblasts, arranged symmetrically in two groups at the posterior edge of the blastodisc, as shown in Diag. 4. These cells have a superficial position at first, but the two median ones are very soon covered by the expanding blastodisc, and the rest are later overgrown by the same elements.

Towards the close of this period, when all arrangements for the formation of the embryo have been completed, free nuclei begin to appear in the surface of the three entoblasts, a, b, c. The origin and fate of these "entoblasts" have been traced with considerable care, and the results of my study on this point have confirmed the opinion that they give rise to the mesenteron.

II. ORIGIN OF THE MESENTERON.

1. *Historical and Comparative.*

Clepsine.—Grube (No. 2), and Leuckart and Rathke (No. 3), derived the entoderm by delamination from the blastoderm. But these authors, whose observations on this subject were made long before the introduction of the microtome, were not able to bring any direct evidence of such a mode of origin. It was simply the most rational conclusion open to them, considering the methods at their command, and what was then known

(2.) GRUBE, A. E. Untersuchungen über die Entwicklung der Clepsinen. *Königsberg.* 1884.

(3.) RATHKE, H., AND LEUCKART, R. Beiträge zur Entwicklungsgeschichte der Hirudineen. *Leipzig.* 1862.

respecting the genetic relations of the germ-layers. Even Hoffmann (No. 4, p. 45), working as late as 1877, with "Pikrocarmin gefärbten Querschnitten," arrives at the same conclusion, and expresses it in words that appear to be, in great part, a direct transcript of Leuckart's phraseology.

According to Robin (No. 5, pp. 297–298), the entoderm arises as a solid cord of cells in the axis of the mass represented by the three entoblasts, *a*, *b*, and *c*. A lumen first appears in the œsophageal portion of the axial cord, and is gradually extended backward, thus forming a digestive tube, with all the yolk lying external to it. Somewhat later (5–8 days after exclusion) the three entoblasts ("globes vitellins") undergo cleavage, breaking up into large cells, that form "*la couche moyenne de l'intestin et particulièrement la couche hepatiqne.*"

In my memoir on "the Embryology of Clepsine" I was unable to give a complete history of the origin of the mesenteron from the three entoblasts, but the facts there presented appeared to leave little room for doubt. Both my observations and conclusions have been contradicted by one of the latest writers on the subject. Soon after the publication of my paper came Hoffmann's (No. 6) second contribution to the embryology of the Hirudinea, in which he declares his opposition to my views in the following words: "Ich muss dieser Entstehungsweise des Darmepithels auf das bestimmteste wiedersprechen. Die 'dark spots in the opaque yolk,' welche nach Whitman in der Zeit, dass die Keimstränge sich ausbilden, so recht deutlich auftreten, sind, ich wiederhole es, nichts anderes als Protoplasma-Masse, welche sich aus dem Deutoplasma neu gebildet hat, sich abschnürt und so an der Bildung der Keimstränge sich betheiligt. *Bei ausgeschlüpften Embryonen findet man innerhalb des Dotters auch nicht einen Kern.* Wären sie vorhanden, so müssten sie an in Pikro-carmin gefärbten Schnitten sichtbar werden, denn die intensiv rothe Farbe der Kerne und des Proto-

(4.) HOFFMANN, C. K. Zur Entwickelungsgeschichte der Clepsinen. *Niederländisches Archiv für Zoologie*, IV., H. 1, p. 31. 1887.

(5.) ROBIN, C. Mémoire sur le Développement Embryogénique des Hirudinées. *Paris.* 1875.

(6.) HOFFMANN, C. K. Untersuchungen über den Bau und die Entwickelungsgeschichte der Hirudineen. *Haarlem.* 1880.

plasmas, wie die grüne der Dotterkörnchen ist zu charakteristisch, als dass man dieselbe übersehen könnte." (p. 53).

"Die Muskelfaserschicht und das Epithelium des Chylusdarmes entstehen beide aus ursprünglich von dem Keimstreifen herrührenden zelligen Elementen" (p. 51). The same origin is claimed for the intestine (Enddarm) and the œsophagus (Schlunddarm).

Metschnikoff (No. 7, p. 672) expresses no decided opinion on the origin of the mesenteron, but throws out the following suggestion. Speaking of the inner stratum (mesoderm) of the germ-bands, he says: "Man kann sehr leicht die Überzeugung gewinnen, dass das sich spaltende Blatt die äussere (*vielleicht auch die innere*) Wand des Mitteldarmes, den sog. Fettkörper, und die Segmentalorgane liefert."

The latest authority on this subject is Joseph Nusbaum (No. 8). In the introduction to his paper, Nusbaum states at some length Hoffmann's erroneous views concerning the nature of the "entoplasts," and then announces, as a discovery of his own, the fact that these entoplasts ("*îlots protoplasmiques*") represent the entoderm. Why so much attention should be lavished on Hoffmann's ideas, which had already been refuted by Bergh, and why the fact should be suppressed that the origin of the entoderm had been ascertained eight years before, I will leave Nusbaum to explain. To discover what has already been discovered, and refute what has already been refuted, is a double-headed offence, inexcusable if the result of ignorance, unpardonable if done deliberately. Nusbaum very narrowly escapes from this charge, for later on he feels constrained to drop the following saving remark: "Selon Whitman, l'épithélium intestinal se forme aux dépens de grands globes entodermiques, à la surface desquels se montrent les noyaux libres et ensuite une couche de cellules épithéliales qui limite de tous les côtés le vitellus nutritif. En un mot, d'après Whitman, l'entoderme secondaire se forme de l'entoderme primitif."

Nusbaum carefully refrains from any direct acknowledgment

(7.) METSCHNIKOFF, ELIAS. Beiträge zur Entwickelungesgeschichte einiger neideren Thiere. Vorläufige Mittheilung. *Mélanges Biologiques* VII., p. 671. 1871.

(8.) NUSBAUM, JOSEPH. Recherches sur l'organogénèse des Hirudinées (Clepsine complanata Sav.). *Archives slaves de Biologie, I. Fasc.* 2, pp. 310–340, *and Fasc.* 3, pp. 539–556. 1886. *Published separate.*

that his conclusions had been anticipated, and turns at once to the more agreeable task of contradicting Hoffmann's account. His own observations are then detailed as follows. Beginning with an embryo in which the somites are already distinctly marked from end to end, and the nephridia differentiated, he says: "Il se montre sur toute la surface externe du vitellus une couche protoplasmique granuleuse (*en*) avec des noyaux ovoides et arrondis; cette couche, développée aux dépens des éléments cellulaires de l'entoderme primitif, se transforme ensuite en épithélium intestinal. Hoffmann a observé aussi une semblable couche protoplasmique avec des noyaux ovoides, mais il l'a considérée comme un produit du mésoderme. Cependant j'ai vu plusieurs fois, sur des coupes très minces et parfaitement colorées, que cette couche est délimitée d'une manière très nette de la couche de cellules plates du feuillet viscéral du mésoderme, tandis que du côté interne la couche protoplasmique passe directement dans le vitellus; ainsi l'origine entodermique de cette couche ne peut être soumise à aucun doute" (p. 9).

This is all that Nusbaum has to tell us about the origin and history of the entoplasts. The whole alimentary tract is lined with cells of the same origin. "Ainsi le résultat final est que l'épithélium (plat) tapissant la cavité de la trompe représente un produit entodermique. Tout l'intestin postérieur représente de même un produit entodermique" (p. 11). The sequel will show that Nusbaum has added very little to what was already known on this subject.

Bergh (No. 9, pp. 259–260) confirms my statements in regard to the existence of peripheral nuclei in the three entoblasts, but rejects the hypothesis that they represent the entoderm, as incompatible with what is known about the origin of this layer in the Gnathobdellidæ.

Nephelis. — According to the observations of Kowalevsky (No. 10, p. 3), Bütschli (No. 11,), and Bergh (No. 9, p. 259), the entoderm in Nephelis arises immediately after the eight-cell

(**9.**) Bergh, R. S. Die Metamorphose von Aulastoma gulo. *Arbeiten a. d. zool.-zoot. Institut in Würzburg.* VII., H. 3, p. 231. 1885.

(**10.**) Kowalevsky, A. Embryologische Studien an Würmern und Arthropoden. *Mém. de l'Acad. Imp. de Sc. St. Pétersburg.* XVI., No. 12. 1871.

(**11.**) Bütschli, O. Entwicklungsgeschichtliche Beiträge. *Zeitschr. f. wiss. Zool.* XXIX., p. 239. 1877.

stage is reached, consisting at first of a few small cells lodged between the micromeres and the macromeres, and derived in all probability from the latter. Bergh is inclined to the belief that this mode of origin holds true of the Rhynchobdellidæ as well as the Gnathobdellidae. He finds in the egg of Clepsine certain cells beneath the blastodisc, in a position which corresponds perfectly to that of the first entoderm cells in Nephelis, and hence infers that they represent the entoderm of Clepsine, although he has not traced their development into this layer.

Notwithstanding the remarkable difference in the position of the "residual" yolk, which distinguishes the two types of leeches represented by Clepsine and Nephelis, it must be taken for granted, so long as no positive proof to the contrary has been produced, that the mesenteron arises in essentially the same manner in both cases. I have ascertained enough of the history of the cells in Clepsine, which Bergh identifies with the entoderm of Nephelis, to convince me that he is partly right on this point; but it is an error to suppose that they constitute the whole, or even the larger part, of the entoderm in Clepsine, and the observations of Kowalevsky and Bütschli fall very far short of establishing such a conclusion in the case of Nephelis. Kowalevsky gives no figures, and offers only the following brief sketch of the germ-layers, which he illustrates by referring to Rathke's plates:—

"Was die Scheidung der Blätter anbetrifft, so ist dieselbe schon auf der Fig. 13, Taf. I., bei Rathke zu entdecken; die oberen Zellen, *ff*, bilden das obere oder *sensorielle* Blatt, die zwischen denselben und den grossen Furchungskugeln liegenden — das *Darmdrüsenblatt* (Figs. 14 and 15), und die drei grossen Kugeln — das *mittlere* Blatt. Weiter umwachsen die Zellen des oberen Blattes die ganze Masse von allen Seiten; die Darmdrüsenblattzellen wachsen sehr schnell, verlieren ihr körniges Aussehen, werden zu grossen hellen Zellen und drängen dabei die grossen Zellen des mittleren Blattes nach hinten, welche durch Abschnürung zwei Zellenreihen bilden, die bekannten Keimstreifen der Nephelis sind." (No. 8, p. 3.)

Kowalevsky's interpretation of the three large segments — the homologues of the entoblasts in Clepsine — as mesoblasts, has been corrected by Bütschli, and his statement on the origin of the entoderm is as indefinite as it is brief.

So far as the published records show, Bütschli is the only one who has thus far undertaken to trace the origin of the entoderm in Nephelis with that precision and attention to details which are now required in such work. But there are some important gaps in his work which must be filled before the origin and relation of the germ-layers can be satisfactorily determined. The posterior macromere in Clepsine, as we have seen, is the sole source of the germ-bands, and its history is the key to subsequent development. Unfortunately our knowledge of this macromere in Nephelis is not complete enough to warrant the assertion that it plays the same rôle; but the facts, so far as they go, point in this direction. When we remember that the germ-bands of Nephelis have been traced by Bergh (No. 10) to ten terminal cells, which we have every reason to suppose are identical with the ten teloblasts of Clepsine, and further, that the eight-cell stage arises in the same manner in both cases, it seems incredible that there should be any radical differences in respect to the fate of the posterior macromere. If, however, this macromere in Nephelis is converted into the ten teloblasts of the germ-bands, — and there is nothing against, and everything for, such a supposition, — it is plain that Bütschli's observations on the origin of the germ-layers are very far from complete. It is simply incredible, in view of what happens in Clepsine, that the whole entoderm and mesoderm should arise in the manner described by him. If the deep cells discovered by Bütschli represent only the earliest and most anterior portion of the entoderm, then the chief difficulty in the way of reconciling the two types of development disappears. This appears to be the only mode of reconciliation open to us, if my observations on Clepsine are correct; and it is entirely permissible to hold this ground until some one has cleared up the history of the posterior macromere in Nephelis.

Robin (No. 5, p. 146) attempted to do this, but his methods of study were not equal to the task, and he fell into the error of supposing that the products of this macromere represented the dorsal moiety of the ectoderm. Bütschli has added but little on this important point; but a summary of his studies on the germ-layers will be needed in order to place the subject in a clear light.

Bütschli's account begins with the eight-cell stage, with what

I have called the second period in Clepsine. This period is initiated by two events, — (1) the cleavage of the posterior macromere, and (2) by the appearance of two small cells beneath the micromeres. Bütschli directs his attention almost wholly to the second of these events, and, with the exception of a few incidental remarks, completely ignores the first.

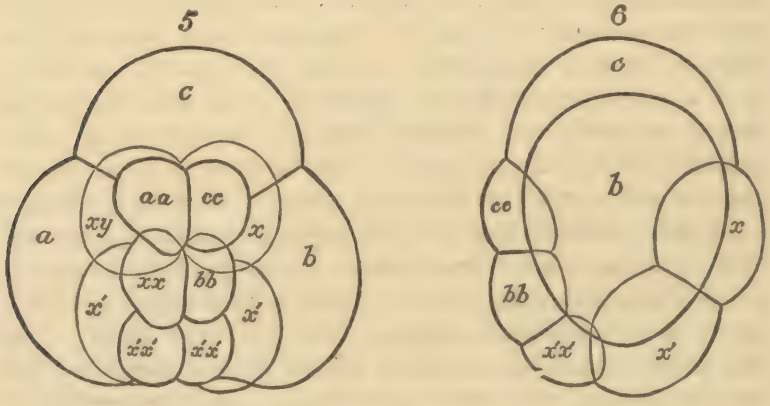
The posterior macromere first divides into two equal parts, one of which occupies nearly a central position on the lower face of the egg, while the other lies behind and slightly above, abutting against the posterior edge of the ectodermic quarter-foil. It will be remembered that similar relations are constant in the corresponding stage of Clepsine, with the single difference that the posterior of the two segments (the neuro-nephroblast) is usually only about one-half the size of the other. It is a fact of considerable importance to us that the second period is introduced in both cases in the same remarkable manner (*i.e.*, by the cleavage of one macromere), for we are thus assured that events are still moving on in parallel courses. I am quite confident that the two segments resulting from the division of the posterior macromere will be found to correspond to the *neuro-nephroblast* and the *primary mesoblast* of Clepsine, and I shall henceforth designate them by these names. One fact only, noticed by Bütschli, throws a little doubt on the identification of the neuro-nephroblast. This segment buds off two micromeres behind the four already formed at the animal pole, and the six micromeres of this stage are arranged in two longitudinal rows.[1] I have seen nothing in the egg of Clepsine comparable with the last two micromeres, but I have shown (No. 1, p. 54, x^4) that two ectoblastic micromeres arise in a little later stage, not directly from the neuro-nephroblast, but from the immediate products of this segment.

The neuro-nephroblast next divides by a sagittal cleavage into two equal parts, which are probably the equivalents of the cells marked x^2 (Fig. 31, Pl. XII.) in my first paper; and then follows the cleavage of the primary mesoblast into two equal parts, bilaterally disposed, and corresponding in position and origin to the two mesoblasts of Clepsine. According to Robin (No. 5, p. 150) the primary mesoblast divides before the neuro-nephroblast, in the same order as in Clepsine.

[1] Precisely like the "mesomeres" in Rhynchelmis.

Possibly the order in Nephelis is variable, in which case Bütschli's observations on this point would appear supplementary, rather than contradictory, to those of Robin.

Bütschli failed to trace the cleavage in detail beyond the sixteen-cell stage, in which (Fig. 5, Pl. XVIII.), as I have shown, every group of segments, and nearly every individual segment even, can be identified with those of the corresponding stage in Clepsine. The close correspondence in parts, relative size, position, and axial orientation, is shown in Diags. 5 and 6. The bulk of the egg is formed of the three macro-



Two views of the sixteen-cell stage of Nephelis. Three deep cells, representing the first entoderm cells, are not shown.

Diag. 5. — Seen as a transparent object from the upper side. (After Bütschli.)

Diag. 6. — Side view of the same stage. (Constructed after Bütschli and Robin.)

a, left macromere; *c*, median and anterior macromere; *b*, right macromere; *aa*, *cc*, *xx*, *bb*, micromeres derived from *a*, *c*, *b*, and *x*.

$x'x'$ and $x'x'$ micromeres derived from the neuro-nephroblast (x'), which is now represented by two cells, x' and x'.

xy, left mesoblast; *x*, right mesoblast.

meres, one (*c*) of which has an anterior median position, while the second and third (*a* and *b*) are laterally placed. The six micromeres are arranged in two bilaterally symmetrical rows, extending from the upper angle of the anterior macromere to the hind edge of the egg. The two mesoblasts (*x* and *xy*) are

symmetrically placed near the middle of the lower face of the egg. Behind and somewhat above them come the two daughter-cells of the neuro-nephroblast. The remaining three cells (not shown) lie beneath the surface, between the two anterior micromeres and the two mesoblasts, and represent the earliest portion of the entoderm. *The embryonic axis has precisely the same relations to the primary-cleavage planes as in Clepsine.*

A further confirmation of the opinion that all the essential details of the second period in the egg of Clepsine repeat themselves in the egg of Nephelis is found in the peculiar shifting of position among the cleavage-products. Bütschli (No. 11, p. 242) briefly alludes to the fact that the smaller cells become more or less completely imbedded in the macromeres, and a glance at his figures shows that the anterior macromere passes backward between the lateral macromeres, and, ultimately, takes a position at the hind end of the embryo. The same movement takes place in Clepsine, only it is not carried quite so far, leaving the macromere in a median ventral position, stretching from end to end.

Bütschli does not discuss the nature of the cells derived from the posterior macromere, and only alludes to them in one place (p. 242) as ectoderm cells. Abandoning the only clue that could guide him safely through subsequent phases, his statements become indefinite, if not obscure; and neither his descriptions nor his figures are free from confusion. Beyond the sixteen-cell stage he is not even able to say whether his figures represent the upper or lower side of the egg, or to state definitely which side corresponds to the ventral or dorsal aspect of the future embryo. Entoderm cells are first represented in red, then in blue, and each of the three germ-layers appears in turn in red. Such incongruity in diagrammatic representation can have but one explanation. Allowing that it was the best that could be done under the circumstances, it is obvious enough that Bütschli failed to leave the subject of the germ-layers in a satisfactory state, and that much remains to be done before the origin of these layers is as clear in Nephelis as in Clepsine.

The three deep cells, which are omitted in the diagrams, were discovered by Bütschli, and interpreted as the "Anlage des Entoderms." Their origin was not directly observed, but their

position was held to be sufficient evidence of derivation from the three macromeres. The number of these cells is very soon raised to six, but whether by renewed proliferation on the part of the macromeres, or by subdivision among themselves, we are not informed. A little later the still more numerous entoderm cells present the form of a solid axial cord, on each side of which is seen a row of three mesoderm cells. These two rows of cells are regarded as the basis of the germ-bands ("Keimstreifen"), but the important question of their origin is left undecided. The mesoderm cells increase rapidly, but not by proliferation on the part of the vitelline macromeres, as supposed by Kowalevsky. Bütschli expressly states that he has never seen these macromeres in process of division, until in a very late stage of the free embryonic life, when, as was first shown by Robin, they break up into a number of cells, which undergo resorption in the body-cavity (Bergh).

In the next stage represented by Bütschli, a narrow, slit-like lumen is seen between the entoderm cells, which is the incipient enteric cavity. The number of entoderm cells must be small, as only *eight* are shown in optical section (Fig. 9, Pl. XVIII.); and it must be noted as a still more remarkable fact that precisely the same number of cells appear in a much later stage (Fig. 12), after the formation of the œsophagus. They have increased immensely in size, while the three macromeres have become proportionately smaller. Bütschli thinks the entoderm cells enlarge at the expense of the fluid food-material contained in the egg-case; but his figures suggest that the growth is at the expense of the macromeres, and analogy would lead one to suppose that the embryo would exhaust its own stock of food-stuff before drawing upon external supplies. It is evident that these large entoderm cells have many changes to undergo before assuming the form of a lining epithelium; but what these changes are, and how the cells multiply, are questions left unanswered by Bütschli's observations. I find no mention of "free nuclei," and no indications of such bodies in his figures, unless the two supernumerary nuclei, shown in Fig. 12, may be so regarded. Such nuclei have been observed in the egg of Nephelis, according to Balfour (No. 12, pp. 3–9).

(12.) Balfour, F. M. A preliminary Account of the Development of the Elasmobranch Fishes. *Quart. Jour. Mic. Sc.* XXII., p. 323. 1874.

"Dr. Kleinenberg has followed a single egg through the whole course of its development, and concludes that the nuclei of Nephelis never become the nuclei of new cells." [1]

Assuming, then, that the ten terminal cells in Nephelis are the homologues of the teloblasts in Clepsine, and that, accordingly, the primitive mesoblastic cords discovered by Bütschli arise neither from the micromeres nor from the three entoblastic macromeres, *a*, *b*, *c*, but from a pair of cells (*x* and *xy*) derived from the posterior macromere, *xx*, the way is clear for comparing the entoderm cells. The chief differences are: (1) the absence of "free nuclei" in Nephelis, and (2) the contra-distinction in position of the three macromeres, which lie *within* the mesenteron in Clepsine, and *external* to it in the body cavity in Nephelis. The foundation of both differences is undoubtedly the relative abundance of food-yolk. The egg of Clepsine is much larger than that of Nephelis, and is completely filled with yolk-spherules, which are to serve as food during several weeks of larval life. The egg of Nephelis, on the other hand, has very little food-yolk, the larva depending upon the fluid albuminous substances contained in the egg-case. The abundance of yolk in the egg of Clepsine makes it necessary for the nuclei to seek a peripheral position, as in the case of so many arthropod eggs; and thus the yolk is left *within* the enteric epithelium. In the egg of Nephelis, the entoderm cells all escape from the macromeres at an early date, and henceforth they multiply by subdivision, and probably grow at the expense

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[1] Balfour has here given Kleinenberg credit for what neither he nor any one else has ever yet accomplished. The method has not yet been discovered by which the egg of Nephelis can be kept alive for more than a few hours, after removal from the egg-case. Continuous observation on a single egg through all its stages of development is, therefore, a feat entirely beyond our present means. Kleinenberg's opinion as to the fate of certain nuclei must rest on evidence of a much less conclusive nature than supposed by Balfour. It is to be hoped that Kleinenberg will yet publish the results of his study on Nephelis, for we certainly stand in need of more light on this subject.

Bergh's brief remark on the origin of the mesenteron may be interpreted in favor of the existence of free nuclei. "Letzteres [Mitteldarmepithel] bildet sich nämlich aus dem primären Entoderm durch *fortdauernde Kernvermehrung;* erst nach dem Ausschlüpfen aus dem Kokon bildet es sich als eigentliches Epithel aus, *indem das Protoplasma sich um die Kerne herum in Zellen sondert.*" (No. 13, p. 294.)

(13.) BERGH, R. S. Ueber die Metamorphose von Nephelis. *Zeitschr. f. wiss. Zool.* XLI. H. 2. p. 284. 1884.

of the macromeres. The earliest entoderm cells are formed in precisely the same manner in Clepsine; but here a few cells are left to multiply in the macromeres, and these take the form of "entoplasts," and are to be regarded as the equivalents of the large mesenteric cells of Nephelis. The œsophagus of Nephelis is probably lined with cells derived from the first-formed entoderm cells, precisely as is the case in Clepsine. *That there is no fundamental distinction between the "entoplasts" of Clepsine and the entoderm cells of Nephelis is shown by the fact that we have both modes of formation in Clepsine, the one passing gradually and insensibly into the other.*

Bergh (No. 9, p. 260) explains the above-named difference in the position of the vitelline macromeres as the result of a retardation in the development of the mesenteron in Clepsine, and apparently regards the Nephelis type of development as the more primitive. The view presented above appears to me more satisfactory. I am not by any means ready to adopt the idea that the mode of development in Clepsine is to be regarded as a modified or derived form of that seen in Nephelis. It would be quite as rational to take just the opposite ground, and maintain that the egg of Nephelis has been derived from one that was heavily loaded with food-yolk. The mode of cleavage, and especially the persistence of the three macromeres, appears to support such a view.[1]

Rhynchelmis (Euaxes).— In spite of the many gaps in our knowledge of the development of Nephelis, it has been easy to find a close and interesting parallel between it and Clepsine in the early phases of the egg. This, to be sure, is no more than might have been expected in the case of two forms so closely allied, but it is more than could have been conceded without first showing how differences of opinion could be reconciled. If now we extend the comparison along the same lines to one of the Chætopods, *e.g.*, Rhynchelmis, we shall find the suggestions advanced in the foregoing pages corroborated in many points of fundamental importance.

[1] The power to elaborate and store nutritive yolk comes and goes with the need; but the mode of development induced by the presence of yolk appears to persist, to a greater or less extent, even after the yolk has been lost. Such has, in all probability, been the case with the mammalian egg, and there is reason to suspect that other alecithal eggs have had a similar history. Many anomalies of development may be accounted for in this way.

The cleavage in Rhynchelmis has been studied with considerable care by Kowalevsky (No. 10, p. 12), and by Vejdovsky (No. 14, p. 228). Some of the changes which the egg undergoes preparatory to cleavage, as described by Vejdovsky, are of great interest, on account of their manifest identity with certain remarkable polar phenomena which display themselves with great intensity in the egg of Clepsine. I refer to the polar rings of hyaline protoplasm, which concentrate at each pole in the form of a disc. The first two cleavage-planes divide the egg into four macromeres, three of which are nearly equal, and correspond to the anterior and the two lateral macromeres of Clepsine, while the fourth and largest one represents the posterior macromere, and contains most of the hyaline protoplasm of the polar discs, precisely as in the egg of Clepsine. Such a close correspondence in the primary steps of cleavage, resulting in the specialization of one of the four macromeres, affords the strongest possible evidence, short of verification by direct observation, that the subsequent history of this macromere will be essentially the same as that of the posterior macromere in Clepsine. Unfortunately, the observations on this point are too incomplete to be decisive; but two important facts may be noted which furnish, at least, a partial verification of the view here taken. First, the median plane of the embryo bears to the first two cleavage-planes relations which are analogous to, if not quite identical with, those described in Clepsine; and, secondly, a pair of mesoblasts arise from the posterior macromere. Remembering that only two teloblasts were hitherto recognized in Lumbricus, and that a careful study of the germ-bands from surface-preparations has led Wilson to the discovery of neuroblasts and nephroblasts, it is not venturing much to predict a similar discovery for Rhynchelmis. In the Hirudinea the mesoblasts lie beneath and usually in front of the remaining teloblasts, and their relations to the germ-bands are not easily ascertained without the aid of sections. Most of the remaining teloblasts (neuroblasts and nephroblasts) are very prominent even in the living egg. In the Oligochæta, on the contrary, the mesoblasts lie behind the other teloblasts, and are conspicuous in surface-views;

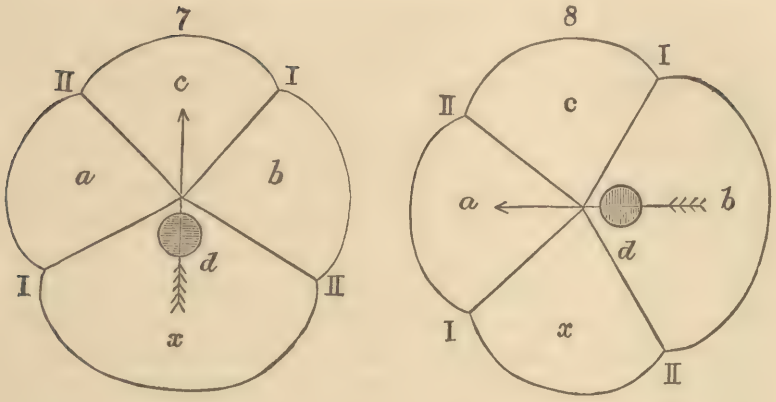
(**14.**) VEJDOVSKY, FR. Die Embryonalentwicklung von Rhynchelmis (Euaxes). Vorluäfige Bemerkungen.

Sitz.-Ber. d. k. böhm. Ges. d. Wiss. March, 1886.

while the neuroblasts and nephroblasts, lying in front of and differing but little in size from the cells which they produce, are discoverable only by careful microscopical examination. This accounts for the fact that they have hitherto been overlooked, and for the widespread belief that the mesoblasts are the sole proliferators of the germ-bands. The origin of the mesenteron, as described by Kowalevsky, is another evidence in favor of identifying the posterior macromere in Rhynchelmis with that of Clepsine.

Let us now consider the history of this macromere more in detail, in order to see how far observation supports the comparison with Clepsine. Vejdovsky has extended and corrected Kowalevsky's account of the cleavage in many particulars, and I shall therefore be guided by his statements.

The first cleavage-plane divides the egg into unequal parts, the larger of which receives the remnants of the polar discs of protoplasm, as in Clepsine. The second cleavage begins first on the larger segment, but passes to the small segment before the large one is completely divided, so that a three-cell stage does not really exist. All this seems at first sight to be in perfect accord with the first two cleavages in Clepsine. But there is one difference in regard to the second cleavage which threatens to upset the whole comparison. This cleavage does not divide the still visible remnant of the polar disc, but runs to the left of them, while in Clepsine it runs to the right. This difference introduces completely new axial relations, analogous to, but not identical with, those in Clepsine, as will be seen by comparing Diags. 7 and 8. It makes 90° difference in the direction of the embryonic axis, for *b* now becomes the posterior macromere, and is destined to play the same rôle as the macromere *x* in Clepsine. The embryonic axis now bisects *a* and *b* (Diag. 8), instead of *c* and *x*, as in Clepsine (Diag. 7). Thus arises a very serious difficulty in the way of identifying the macromeres. I see only two ways of meeting this point. 1. There is, of course, a possibility of error in observation, and the liability to error is all the greater, as Vejdovsky does not appear to have given any attention to the relations we are now considering. 2. If he is right in placing the second cleavage-plane to the left of the disc, it will still be possible to identify the macromeres, *provided the order of the first two cleavage-*



Diagrams of the four-cell stage of Clepsine and Rhynchelmis (Euaxes) to show the relations of the cleavage-planes to the median plane of the embryo.

DIAG. 7. — Clepsine. The axis of the embryo bisects two opposite macromeres (*c* and *x*).

DIAG. 8. — Rhynchelmis (constructed after Vejdovsky). The embryonic axis here bisects opposite macromeres, but stands at right angles to the position shown in DIAG. 7.

a, *c*, *b*, *x*, the four macromeres.

d, the polar disc of hyaline protoplasm, which marks the posterior macromere.

I – I, first cleavage-plane; II – II, second cleavage-plane.

planes in Rhynchelmis is the reverse of that in Clepsine. That is, if the first and second cleavage-planes in Rhynchelmis correspond respectively, to the second and first in Clepsine, there will be a complete correspondence of macromeres and axial relations in the two eggs. The appearances are so strongly in favor of the identity of the posterior macromeres, that either alternative appears to me more acceptable than the conclusion that there is a radical difference in axial relations that bear all the outward marks of being absolutely identical. I shall proceed on the assumption that the difficulty just considered arises from an error of observation, and that the relations between the median plane of the embryo and the cleavage-planes are precisely the same in both cases. Future observation must determine whether this position is well chosen.

The cleavage of the posterior macromere is described by Vejdovsky in the following words: —

" In den nachfolgenden Vorgängen spielt die hintere Makromere die wichtigste Rolle, in der, wie bemerkt, das Protoplasma

von beiden Polen sich concentrirte. Nachdem nämlich die ersten 4 Mikromeren ihre definitive und gleiche Grösse erlangt haben, knospet aus der hinteren Makromere eine grössere, aus Protoplasma bestehende Zelle, die, wie die vertikalen Längsschnitte zeigen, aus dem gemeinsamen Protoplasmanest ihren Ursprung hat und bezüglich der Grösse zwischen der des Mikromeren und Makromeren steht; somit werden wir sie als *Mesomere* bezeichnen.

"Dieselbe verdrängt die inzwischen vermehrten Mikromeren mehr nach vorne und bald darnach bildet sich in derselben Weise aus der hinteren Makromere, beziehungsweise aus deren Protoplasma die *zweite Mesomere*, die sich hinter der ersten stellt, und schliesslich entsteht die *dritte Mesomere*, welche in Bezug auf die Grösse und Gestalt den vorderen zwei vollständig gleich ist. Mikromeren sind bereits zahlreich zu beiden Seiten und nach vorne vorhanden. Die Längsschnitte durch dieses Stadium beweisen, dass *die hintere Makromere bereits des Protoplasma entbehrt, indem dasselbe zur Bildung der Mesomeren verwendet wurde.*

"Die vorderen zwei Mesomeren bleiben eine Zeit lang unverändert, während die dritte, hintere Mesomere sich mehr der Quere nach ausbreitet und schliesslich sich in zwei neue, gleich grosse Mesomeren theilt, die bald zu der ursprünglichen Grösse ihrer Mutterzelle heranwachsen. Bald darnach theilen sich in der Längsaxe auch die zwei vorderen Mesomeren, so dass *zwei Reihen von je drei gleich grossen Mesomeren entstehen*, die aus der Umgebung der inzwischen stark sich vermehrenden Mikromeren hervortreten. Die vorderen zwei Mesomeren theilen sich nun — immer in der Längsaxe — in zwei, dann in vier Zellen, die aber nicht wachsen, sondern durch weitere Theilung die Grösse der Mikromeren annehmen. An solchen Eiern treten nur die vier hinteren Mesomeren hervor. Dieselben Theilungsvorgänge wiederholen sich aber bald auch an dem zweiten Paare der jetzt vorderen Mesomeren und somit zerfallen dieselben in eine Anzahl der Mikromeren, während *das hinterste Paar der Mesomeren sowohl ietzt als auch später bei der späteren Furchung der Makromeren unverändert bleiben und als zwei weisse, stark gewölbte Kugeln dem hinteren Ende der Mikromeren aufsitzen.*" (No. 14, pp. 234–335).

It is evident from this description that Vejdovsky's "meso-

meres" correspond to the teloblasts of Clepsine. The two posterior mesomeres represent the mesoblasts, while the four anterior mesomeres probably represent the neuroblasts and the nephroblasts. Vejdovsky lets the anterior mesomeres divide up into micromeres, and fails to connect them with the nerve-cord and the nephridia. The discovery of a full set of teloblasts in Lumbricus makes it almost certain that such cells are concerned in the formation of the germ-bands of Rhynchelmis, and the mesomeres appear to be the only cells that could here be so identified. Allowing that the mesomeres admit of this interpretation, it is clear that the posterior macromere in Rhynchelmis fulfils the same ends as does the posterior macromere in Clepsine. The mesomeres differ from the teloblasts in number and in mode of origin, but agree with them in position, derivation, and purpose.

According to Vejdovsky (p. 235), the remnant of the posterior macromere, after the production of the three primary mesomeres, takes part with the other three macromeres in the formation of the mesenteron. I have not traced any entodermic elements to this macromere in Clepsine; but, in view of the fact that the mesoblasts persist for some time after they cease to contribute to the germ-bands, it would not be strange to find that their final products were entoplasts. Should this turn out to be the case, the origin of all the germ-layers would be as nearly the same in both eggs as one could well expect.

The origin of the mesenteron in Rhynchelmis has been well described and figured by Kowalevsky. A glance at his figures is sufficient to show that the mode of origin is essentially the same as in Clepsine. There are three primary entoblasts (four with the remnant of the posterior macromere), differing from those in Clepsine only in breaking up into a number of secondary entoblasts. The formation of entoplasts takes place in the same manner as in Clepsine, and the residual yolk is finally inclosed in the mesenteron. The whole process is so similar to what I have described in Clepsine that I have been surprised to find Hoffmann and Bergh, who must be familiar with the facts, disposed to reject my conclusions. It is hardly necessary to add that this mode of origin is very common among Arthropods; and that here, as in Rhynchelmis, the

similarity extends to the œsophageal portion of the alimentary canal.

It is an interesting fact that in those cases where the major portion of the mesenteron passes through well-marked stages of differentiation, beginning with one or more primary vitelline spheres, then, with or without further subdivision of these spheres, taking the form of free peripheral nuclei imbedded in protoplasm with no defined cell boundary-lines (entoplasts), and finally assuming the form of a distinct lining epithelium; there is always an anterior portion, which arises quite early, and which, from the first, consists of distinct cells, often scarcely distinguishable from the cells of the ectoderm and mesoderm. Numerous cases might be cited, but I must here limit the comparison to Rhynchelmis. On this point I may refer again to Vejdovsky. Speaking of the origin of the entoderm at the anterior end of the embryo, he makes the following remarks: —

"Es bildet sich hier eine Gruppe dicht neben und aneinander liegenden Hypoblastzellen, *die der Dotterkügelchen völlig entbehren und sich ebenso intensiv roth wie die Keimstreifzellen tingiren. Den ersten Anfang derartiger Hypoblastzellen kann man bereits in dem Stadium finden, als die Mesomeren sich einzustülpen*[1] *und die ersten Keimstreifzellen zu produciren beginnen. Aus den derart differencirten Hypoblastzellen bildet sich später die Epithelschicht des Anfangstheiles vom Mitteldarme — der Oesophagus.* (No. 14, p. 237).

Branchiobdella. — In a recent paper on the embryology of Branchiobdella, Salensky (No. 15, p. 1) has compared this form with Clepsine and Nephelis. The paper is a most welcome addition to our knowledge of this interesting parasite; but the points of chief interest here — the cleavage, axial relations, and origin of the germ-layers — are too imperfectly worked out to admit of a close comparison with Clepsine.

Salensky finds on one pole of the egg a clear spot, which

[1] The "Einstulpung" of the mesomeres, described by Vejdovsky, is comparable with the movements which result in imbedding the teloblasts of Clepsine in the vitelline spheres. I believe this imbedding process is best understood as a part of the invaginatory movement described in my former paper (No. 1, pp. 58–59). The mesoblasts lie at the hind end of the blastopore, *between* the entoblast and the ectoblast, and their products are carried inward between the two primary layers.

(15.) SALENSKY, W. Développement de Branchiobdella. *Arch. de Biol.* vi. *Fasc.* 1, p. 1. 1885.

corresponds in position to the archiamphiaster ("amphiaster de rebut"), and which is traversed by the first cleavage-groove. This pole is correctly identified with what I have called the "oral pole" in Clepsine. In view of these facts it is very remarkable that Salensky should regard the first cleavage-groove as equatorial. I fail to see a single fact which could be urged in support of such a view. The relation of this groove to the "clear spot" is conclusive evidence that it is meridian, precisely as it is in the Hirudinea and other annelids. If further proof be required, it may be found in the development of the eight-cell stage, which corresponds in every prominent feature with the same stage in Clepsine, Nephelis, and Rhynchlemis. This is a point of primary importance; for if the first cleavage were equatorial, the subsequent cleavages would be just as little comparable with those of Clepsine as the first, and the axial orientation would be radically different in the two cases. Precisely how the embryonic axis is related to the first two cleavage-planes is not clear; but, judging from Salensky's figures, it appears to be the same as that of the types before considered. There is a large macromere, from which the first micromere arises, and which divides, asymmetrically, prior to the division of the other three macromeres. It is this macromere that I would identify with the "posterior macromere" in Clepsine. According to Salensky (pp. 20–21) the two segments into which this macromere divides, participate equally in the formation of all the germ-layers. That is, they are not destined to play unlike parts, as they do in Clepsine, the one, representing a primary mesoblast, the other a neuro-nephroblast, but each alike gives rise to entodermic, mesodermic, and probably ectodermic elements. "Elles donnent naissance aux cellules *mésoentodermiques* et ne représentent point des ébauches spéciales ni du mésoderme, ni du système nerveux, comme c'est le cas chez Clepsine."

There appears to be the same confusion here as we found in Nephelis, in regard to the origin of the entoderm and mesoderm. The earlier "meso-entodermic" cells in Branchiobdella appear to correspond to the first deep cells which arise beneath the micromeres in Clepsine and Nephelis, and which, as I have shown, are purely entodermic. Salensky has found cells which are unmistakably the homologues of the teloblasts of Clepsine; but he has failed to trace their origin and to deter-

mine the special part which each plays in the formation of the embryo. Although he has thus left it impossible to distinguish the different kinds of teloblasts, we may safely assume that they represent mesoblasts, neuroblasts, and nephroblasts, and that they all originate from the large posterior macromere. Allowing that the teloblasts are here similar in origin and character to the teloblasts of Clepsine, Nephelis, and Lumbricus, it follows that the entoderm must arise, chiefly at least, from the other three macromeres. The chief difference, then, between Branchiobdella and Clepsine, in respect to the entoderm, would lie not in the *source*, but in the *mode* of origin. In the former the cleavage process is continued to the end, while in the latter it ceases with the formation of the primary macromeres, the work then being completed by the intermediation of entoplasts.

In regard to the extent to which the epithelium of the alimentary canal is of entodermic origin, Salensky remarks (p. 56): "Ce canal tout entier, à l'exception des parties insignifiantes avoisinant la bouche et l'anus, est exclusivement formé par l'entoderme." Speaking of the œsophagus, he says (p. 58): "Le processus de l'évolution de l'oesophage, chez Branchiobdella, démontre clairement que tout l'épithélium de cette partie nait *exclusivement* aux dépens de l'entoderme." The same will be shown to hold true in Clepsine.

Salensky (No. 15, p. 19) has misunderstood my statements with reference to the relation of the embryonic axis to the main axis of the egg. The cephalic lobe is very nearly centred on the upper pole of the egg in Clepsine, and the mouth arises at, or at least very near, this pole. It does not follow, however, that because the mouth is located at the oral pole, the posterior end of the embryo must be found at the opposite, or aboral pole. A glance at my figures will show that the two ends of the embryo are at first very near together, the caudal end itself lying just behind the area occupied by the four primary micromeres. The axis of the embryo may, therefore, be said to be at right angles to that of the egg, as it is in Branchiobdella and Nephelis.[1]

[1] I cannot agree with Salensky that the embryology of Branchiobdella establishes its title to be ranked among the Hirudinea. Both its development and adult structure appear to me to sustain the opinion, now held by most authorities, that it stands nearer the Oligochæta than the Hirudinea.

2. *Observations.*

The results of my study on Clepsine parasita are supplementary to those obtained on C. marginata (No. 1, pp. 57–58, 66–72) and C. complanata. For the sake of clearness as well as completeness, I have decided to give both the earlier and the later observations a place in the present paper. The existence of free nuclei in the surface of the three entoblasts, *a*, *b*, *c*, first pointed out in my paper on Clepsine, has been confirmed by Bergh and Nusbaum. The early history of these nuclei is given in the following citation: —

"About the time the germ-bands begin to form, a number of free nuclei appear in the surface of the entodermal blastomeres, *a*, *b*, *c*. These nuclei are very distinct in the egg of C. complanata, and it is remarkable that they have so long escaped observation. They appear like dark spots in the opaque yolk, just as the nuclei of the neuroblasts or of the blastodisc. They are oval, oblong, or biscuit-shaped, and measure .02 to .05 mm. At the time of appearance they number three to four in each blastomere, two or three of which occupy the position seen in the figure (No. 1, Fig. 37), while the others are near the lower pole. They are encircled by white rings, such as are generally seen around the nuclei of the neuroblasts. The substance of these rings is the same as that of the white borders of the rings and ring-discs.

"I have seen these nuclei pass through the successive forms of a dividing amphiaster. They multiply rapidly, and, in the stage of Fig. 38, are scattered over the whole outer surface of the blastomeres. In the following stages they can also be seen on the upper faces of *a*, *c*, and *b*, through the thin ectodermal layer. By the time the germ-bands are fully united they are very numerous, and much smaller than at first.

"Whence come these nuclei? In the stage of Fig. 35 they are not to be seen. A horizontal section of this stage (Fig. 80) shows that each blastomere possesses a single nucleus. The nucleoplasm has a somewhat stellate form. The rays vary in length, sometimes reaching to the irregular circular outline of the nucleus. The same condition has been described in Nephelis by Bütschli. Fig. 61 represents one of these nuclei in a little earlier phase. The nuclei now lie nearer the inner

than the outer faces. Fig. 83 represents a horizontal section of the stage of Fig. 37, which passes beneath the neuroblasts and the blastodisc. Here only two nuclei were hit, but these lie near the outer faces of the blastomeres. *The nuclei of the blastomeres then pass from their original central position to the periphery*, and can here be seen in the living egg." (No. 1, pp. 57–58.)

I was unable to trace these nuclei directly through all their later stages of multiplication, but various facts led me to conclude that they gave rise to the mesenteron. Between the last stage in which I could recognize these nuclei and that in which the mesenteron became distinct, a number of stages intervened in which I was unable to demonstrate their existence. This failure, due to imperfections in methods of preparation, was a source of doubt, in spite of the many indications which made it appear almost certain that they represented the mesenteron. I satisfied myself that the entoderm could not have its origin in elements derived from the germ-bands, for all these elements were plainly turned to other uses. Besides, the earliest appearance of the entoderm cells, — their loose and irregular order *in the periphery of the yolk*, — pointed to their origin from the free superficial nuclei of earlier stages. After describing the earliest appearance of the mesenteron (p. 66), I stated my conclusion in the following words: —

"These superficial nuclei go on multiplying by division during the whole period of the epiboly. Finally they are seen as mere white dots scattered over the entire surface of the yolk. Six to seven days after exclusion the entoderm cells make their appearance as *clear cells* with small nuclei, in the periphery of the yolk already cut up into compartments by the septa. What hypothesis is more probable than that these cells originate from the free nuclei? My sections have convinced me that *these entoderm cells arise in the surface of the yolk, and that they do not originate in the products of the blastodisc*" (p. 67).

The condition of the mesenteron represented in Figs. 93 and 95 of my former paper, is very similar to that shown in Nusbaum's Figs. 13 and 14, Pl. II.

With regard to the origin of the free nuclei from the primary nuclei of the entoblasts, I have nothing to add to the statements above cited. The observations which follow begin with

an early stage of the embryo, before the germ-bands have reached the equator of the egg, and the illustrations for the earlier phases described (Figs. 1–5) are drawn from eggs of C. complanata, obtained at Naples.

Figure 1 represents a surface view of the egg, with the germ-bands in an equatorial position, before they have united at the cephalic end. The white patches (*enp*) seen in the yolk, below the germ-bands, are *nucleated* masses of finely granular protoplasm, to which I have given the name *entoplasts*. The existence of such bodies was entirely overlooked by Hoffmann in his first paper; but in his second paper he describes them as "*Protoplasmaflecke*," and devotes considerable space to refuting my interpretation of them. Nusbaum (No. 8, p. 2) carefully translates Hoffmann's description, as if it were something original, using "*îlots protoplasmiques*" as the equivalent of Hoffmann's term.

Sections of the egg (Figs. 2–5) show that many of the entoplasts have not yet reached a peripheral position. In a sagittal section of the head (Fig. 4) we see scattered entoplasts (*enp*) in the yolk, and external to them some large entoderm cells (*en*). Some of these cells are only faintly or imperfectly circumscribed by boundary lines, representing transitional phases between the entoplast and the clearly defined entoderm cell. The differentiation of the entoplasts is going on more rapidly in this region than elsewhere, and it is here that they first assume an epithelial character. Another sagittal section of the same egg, nearer the median plane, is seen in Fig. 3. Here the same large entoderm cells are seen beneath the head (*cl*), and a few neighboring entoplasts, not yet delimited, against the yolk. This one section shows fourteen entoplasts, only three of which could have been seen from the surface. The rest lie beneath the cephalic lobe (*cl*) and around the mesoblast *x*, which is here nearer the anterior than the posterior end of the egg. In the transverse section (Fig. 2) fewer entoplasts are seen, — four in *a*, three in *c*, and one in *b*. The protoplasm surrounding the two nuclei at the upper angle of *c* is continuous with the ectoblast, which raises the question whether contributions to the latter have continued up to so late a stage. I am not able to decide the question, but I am inclined to think that the macromeres cease to proliferate ectoblastic elements

when the formation of free nuclei begins. Near the upper angle of *xy*, between *xy* and *a*, is seen a well-defined entoderm cell. It is rare to find the entoplasts assuming the cell form at such a depth. Fig. 5 (a horizontal section in the plane of the arrow 5–5, Fig. 3) shows entoplasts at different depths, one of which has become defined as a cell (*en*), while another near by shows faint indications of its future outline.

Passing now to the stage in which the germ-bands have united for about one-half their length, we find all the entoplasts in the periphery of the yolk, and a marked advancement already made in the development of the cephalic end. Beneath the stomodæal thickening (Fig. 20, Pl. VI.) is seen a mass of large clear cells (*en*), as yet presenting no definite form and giving no indication of their future histological character. They are easily identified with the entoderm cells beneath the cephalic lobe in Figs. 3 and 4.

In a little later stage (Fig. 21, *en*), when the germ-bands are nearly closed, these cells are smaller, and appear to be taking a more definite shape, as they become more sharply delimited from mesodermic (*m*) and neural (*sup*, *oe*, *g*) elements. We now distinguish an anterior axial portion, forming a solid pad beneath the stomodæum, and a posterior portion (*sgl*), consisting of larger cells, stretching towards the dorsal and ventral sides. Those of the dorsal side are more deeply stained than those of the ventral side. In several instances I have seen a column of these clear cells extending through the centre of the stomodæum, as shown in Fig. 21; and this leads me to believe that the canal of the proboscis is lined throughout with cells of entodermal origin. The condition shown in Fig. 28 fully bears out such a view. Along the ventral side of the yolk (Fig. 22) there is a peripheral layer of coarsely granular protoplasm ("conche protoplasmique granuleuse" of Nusbaum), feebly stained, in which may be seen free nuclei (*enp*). Similar nuclei are found on the dorsal side, but there they are less numerous, and the peripheral layer of granular, uncolored protoplasm is not present.

By the time the germ-bands are completely closed, and the embryo is ready to leave the egg membrane (Fig. 28), the entoderm of the œsophageal region has differentiated into small, axially placed cells, of a distinctly epithelial character, and

larger cells, destined to form the several pairs of massive cell-groups, known as the salivary glands (*sgl*), which are later found on the dorsal and ventral sides of the pharynx, with canals leading into the extreme hind end of the proboscis. The epithelial portion extends through the proboscis, now distinctly marked off from the rest of the stomodæum, and reaches backward between the dorsal and ventral masses of glandular cells. There is still no lumen recognizable in any portion of the œsophagus, but the entodermal epithelium is so well differentiated in color and general appearance, and agrees so perfectly with the conditions seen later in the posterior portions of the alimentary canal, that there is not the least difficulty in distinguishing it from the other embryonic tissues associated with it.

On the dorsal side of the yolk the entoderm is still represented by entoplasts (*enp*), but on the ventral side, by an extremely thin layer of epithelial cells (*en*), which would be easily overlooked, except for the strongly stained oval nuclei. In my study of C. marginata I failed to recognize this very obscure layer, and thus overlooked a stage of development which connects the entoplasts with the epithelium derived from them. The same condition is seen along the middle region of the ventral side, as shown in Fig. 26, *en*, but the layer vanishes a little farther on, behind which point we find scattered entoplasts (Fig. 27, *enp*). The gradual transition from this flattened epithelium into columnar epithelium is well shown in Fig. 25, which represents the dorsal half of one of the anterior cæca in C. marginata, nine days after hatching.

Recapitulation. — The history of the mesenteron may now be recapitulated.

1. The earlier entoderm cells arise beneath the cephalic lobe, and are probably budded off from the entoblasts, *a*, *b*, *c*, as distinct cells, precisely as in Nephelis. But to these earlier and regularly formed cells are soon added others, which appear first as entoplasts, so that it is impossible to draw any line of distinction based on the mode of origin.

2. The larger portion of the mesenteron, embracing the whole alimentary tract, with the exception of a small, anterior (œsophageal) portion, passes through the following stages of development. The first stage is represented by the *three large macromeres*, or *entoblasts* (*a*, *b*, *c*); the second by *entoplasts*

(each represented by a nucleated mass of protoplasm without cell-boundary); the third by an exceedingly thin layer of *flattened epithelium;* and the fourth by a *columnar epithelium.*

3. The development of the mesenteron begins at the anterior end, and progresses towards the posterior end, but more rapidly along the ventral than the dorsal side.

4. The phases of development are essentially the same as in Rhynchelmis, the chief difference being that, in the latter, the three primary entoblasts *a*, *b*, *c*, split up into secondary entoblasts before the entoplastic phase appears.

5. The history of the mesenteron in Nephelis is very imperfectly known, but there is nothing in the observations thus far published which appears to be irreconcilable with the results obtained in Clepsine. The development in Clepsine is more complicated, owing to the larger amount of food-yolk. It is doubtful whether the entoplastic phase is represented in Nephelis.

6. It is possible that the residual mesoblasts (the remnants left after the completion of the germ-bands) contribute to the formation of the mesenteron. Such a termination of their history has not been ascertained, but is suggested by the fate of the posterior macromere in Rhynchelmis.

7. The proboscis — the homologue of the muscular pharynx of the Gnathobdellidæ — is lined with cells of entodermal origin. The rest of the proboscis, together with the proboscidial or pharyngeal pocket, is derived from the stomodæal thickening of the ectoderm.

8. A knowledge of the history of the teloblasts clears up many obscurities in regard to the origin and relations of the germ-layers, particularly the entoderm and mesoderm. If the precise origin of the teloblasts be known, that of the entoderm may be inferred, and *vice versâ*.

III. THE ECTODERM AND ITS PRODUCTS.

1. *The Ectoderm.*

The origin of the first four ectodermic cells (micromeres) has been described under the head of cleavage and axial relations. By the addition of numerous other micromeres, arising, mainly, from the anterior and the lateral macromeres, a sort of blastodisc is gradually formed, centered at the upper pole of the egg. This blastodisc is not wholly ectodermic, for a few of its deeper cells, as we have seen, represent the earlier entoderm cells, as was first suggested by Bergh. The superficial, ectodermic portion of the blastodisc gives rise to the epidermal layer and its derivatives, the stomodæum, sense-organs, etc.

The ectoderm includes, in addition to the superficial portion of the blastodisc, all the teloblasts, except the two larger and and deeper ones, which represent mesoblasts. The grounds for regarding the eight smaller teloblasts as part of the ectoderm are the following: 1. They have at the outset a superficial position at the hind edge of the blastodisc. 2. Two of them give rise to the ventral nerve-cord. 3. In Lumbricus (*vide* Wilson) they lie in, and plainly form a part of, the general ectoderm.

2. *The Ventral Nerve-chain.*

In a preliminary paper (No. 16) I have already stated that the nerve-chain of Clepsine first appears in the form of two simple, unsegmented rows of cells; and, further, that each row is the product of a single cell, the neuroblast. At the time this fact was announced nothing of the kind was known in any other animal; and Nusbaum, the latest authority on Clepsine, had just arrived at an entirely different conclusion, and one altogether more in harmony with traditional views. A similar discovery has since been made by Wilson in Lumbricus, and, fortunately, the evidences in both cases can now be presented side by side. The subject is one which has received a good deal of attention, and given rise to considerable discussion. Before reviewing the opinions of other writers, or giving my

(16.) WHITMAN, C. O. The Germ-Layers of Clepsine. *Zool. Anz.* No. 218. 1886.

own observations, it will be well to consider briefly some points respecting the germ-bands.

Use of the Term Germ-bands. — It is important to settle at the outset precisely what is to be understood by the term "germ-bands." Keimstreifen, the German equivalent, is usually restricted to the strata derived from the teloblasts, the epidermal layer being excluded. It is, furthermore, generally believed that the germ-bands of the annelids embrace only mesoblastic elements. Although appearances may often favor such a restricted use of the term, we cannot so limit it in all cases. The idea that the germ-bands are purely mesoblastic has already led to much confusion. In the Hirudinea it is perfectly certain that ectoblastic elements must be included, and hence the matter is not in the least simplified by excluding the epidermal stratum. In Lumbricus, where, according to Wilson, the neuroblasts and nephroblasts are at first ordinary ectoblastic cells, and where, after sinking beneath the surface, they remain imbedded in the epidermal layer, it is obvious that this layer cannot well be held to be distinct from the elements of the germ-bands. According to Bergh (No. 9) the "definitive epidermis" in the Gnathobdellidæ arises from what I shall call the *neuronephric* stratum of the germ-bands. I cannot, therefore, follow Kowalevsky, Bütschli, Hatschek, Balfour, Goette, and numerous other writers in the use of "mesoblastic bands" as the equivalent of germ-bands, nor can I accept the alternative offered by Bergh (No. 9, p. 285), which denies the homology of the germ-bands. The moment we undertake to exclude ectodermic elements, the basis for homology is sacrificed, and the door is open to endless confusion. I shall, therefore, include in the term germ-bands both the "mesoblastic bands" and the superjacent ectodermic strata. It must be remembered, also, that the term, as here used, has reference only to the body of the embryo.

Germ-bands of the Head. — The relations of the cephalic lobe to the germ-bands have not yet been made clear in Clepsine. In Aulostoma, Bergh (No. 9) finds in the head two distinct germ-bands, which arise independently of each other and of the germ-bands of the body. The head-bands ("Kopfkeime") contain epidermal, neural, and muscular elements, and are regarded as homodynamous with the trunk-bands ("Rumpf-

keime"). Leuckart (No. 17, p. 706) described the head-bands in Hirudo as "Zwei einfache seitliche Anschwellungen, die rechts und links vor der Mundöffnung gelegen sind und durch eine ziemlich lange Commissur sowohl unter sich, als auch mit den jetzt hornförmig ausgezogenen Vorderenden der Unterschlundganglienmasse zusammenhängen." Semper (No. 18, p. 215) was the first to point out two distinct head-bands ("Sinnesplatten," "Kopfkeimstreifen") in the Hirudinea. "Bei Clepsine, wie bei Nephelis, der Schlundring und das dorsale Schlundganglion entsteht gerade so wie bei Hirudo, durch Verwachsen zweier Sinnesplatten." He insists, however, that these sense-plates (p. 247) are "echte Kopfkeimstreifen, von deren Bildungszellmasse nur ein Theil zum Nervensystem wird, während ein anderer Theil sich in die übrigen Organe des Kopfes, vor Allem in die mit dem Schlunde sich verbindenden Organe umwandelt."

As above remarked, I am not prepared to discuss the question as to the existence of two independent germ-bands in the cephalic lobe. I have stated (No. 1) my conviction that this lobe is formed at the expense of the first four micromeres. Should there prove to be two head-bands, as maintained by Semper and Bergh, it would be an interesting problem to determine whether they hold the same relation to the first four micromeres as the germ-bands of the trunk to the teloblasts. If such a relation could be demonstrated, the homodynamy of head and trunk would be placed in a very instructive light. The entire embryo, with exception of the mesenteron and epidermis, would then be built up in fundamentally the same manner, at the expense of terminal blastomeres, ten teloblasts, and four micromeres, or acroblasts. The theoretical difficulty presented by independent rudiments for the head and trunk could then be disposed of. The temporary separation of the rudiments might be regarded as an accident of their present mode of origin rather than as an expression of their primitive relations. Their real and essential unity is discoverable in the history of the originating blastomeres. It will be remembered that the posterior macromere produces not only the mesoblasts, neuroblasts,

(**17.**) Leuckart, R. Die Menschlichen Parasiten. I. 1863.

(**18.**) Semper, C. Die Verwandtschaftsbeziehungen der gegliederten Thiere. *Arb. a. d. Zool.-Zoot. Inst. in Würzburg.* III. 1876.

and nephroblasts, but also one of the four primary micromeres. The teloblasts stand thus in the direct line of descent with the acroblasts, and are at first in close contact with them. The full significance of the teloblasts and their original relations can only be made clear by comparison with the larval forms of other annelids. Farther on I shall indicate briefly some points in this comparison.

Germ-bands of the Trunk. — Each germ-band consists of three distinct layers: (1) A thin epidermal layer, (2) a neuro-nephric layer, and (3) a mesoblastic layer. The character and relative positions of these layers may be seen in Figs. 2, 6, and 7, Pl. IV. The epidermal layer (*ep*) consists of flattened cells, more deeply stained with osmic acid than the underlying strata. The neuro-nephric layer is represented by four longitudinal rows of cubical or oval cells (*nc*, *nph*, and *m'*), as is best seen in surface views (Fig. 8, Pl. V.). The mesoblastic layer (*m*) consists of large, rounded, or polygonal cells, two or more deep, filling the space between the neuro-nephric layer and the yolk.

Origin of the Ventral Nerve-Chain. — As my observations on the origin of the nerve-chain contradict those of Kowalevsky and Nusbaum, and as they do not confirm the anticipations of such clear-sighted embryologists as Balfour, I can hardly do justice to the subject without dealing briefly with its historical side.

(*a*) *Historical and Critical.* — Filippi (No. 19, p. 23), the earliest writer on the embryology of Clepsine, tells us that it is impossible to trace the origin of individual organs, owing to the small size of the embryos.

Grube (No. 2, p. 35) derived the nerve-cord from the germ-bands ("Bauchwülsten"), the defective technique of the times not enabling him to reach more definite results.

In the posthumous work of Rathke, edited and revised by Leuckart, the nerve-chain is said to be formed from the median part of the germ-bands ("Bauchplatten"). This result, obtained long before the introduction of the microtome, comes much nearer the truth than the statements of Kowalevsky or Nusbaum. Without the aid of sections, the inner stratum of the

(19.) FILIPPI, F. DE. Sopra l'Anatomia e lo Sviluppo delle Clepsine. *Pavia*, 1839.

germ-bands could not be distinguished from the neuro-nephric stratum, and this is the principal failure in Rathke and Leuckart's description. They recognized four rows of cells, each terminated by a teloblast, but were mistaken in supposing that these rows alone made up the entire thickness of the bands. Their conclusion is stated in the following words (No. 3, p. 94): "Wie bei Nephelis, so scheidet sich auch bei den Clepsinen ein jedes dieser Täfelchen [metamere] in zwei neben einander liegende Hälften, von denen die eine, *die der Medianlinie des Körpers anliegt, zu einem Theile des Bauchmarkes wird*, während sich die andere in ein plattes und dünnes Bündel quer verlaufender Muskelfasern entwickelt."

In Leuckart's celebrated work on Human Parasites (No. 17, pp. 702–703), the longitudinal commissures and the lateral ganglia of the nerve-chain are described (in Hirudo) as arising independently of each other, the ganglia being formed from the median portions of the bands, while the commissures are derived from a "helle Furche" (presumably ectodermic) which separates the bands. "Der Primitivstreifen besteht aus zwei Hälften, die trotz ihrer dichten Anlagerung in der Mittellinie durch einen schmalen Zwischenraum getrennt sind. Bei durchfallendem Lichte erkennt man hier eine *helle Furche*, die ziemlich bald, von den sich rasch entwickelnden Längsfasern, ein etwas streifiges Aussehen annimmt."

The origin of the ganglia from the median parts of the germbands, subsequent to the division into metameres, and of the commissures from the median groove, is then described as follows: "Die Entwicklung der Ganglien geht von dem Innenrande der einzelnen Felder [metameres] aus und geschieht dadurch, *dass dieser zapfenförmig in die Längsfurche zwischen den beiden Hälften des Primitivstreifens hineinwächst, sich an den hier, wie erwähnt, schon früher vorhandenen Längsfaserstrang anlegt und schliesslich von der übrigen Zellenmasse des Feldes abtrennt.*"

Metschnikoff (No. 7, pp. 671–673) was the first to recognize three distinct strata in the germ-bands, and also the first to determine the origin of the nerve-chain from the neuro-nephric stratum. He was wrong, however, in supposing that this whole stratum is converted into the nerve-cord. His brief description runs thus: "Bei dem ersten Erscheinen der beiden Keimstreifen bestanden dieselben bereits aus drei Keimblättern. Das oberste

Blatt erschien in Form eines dünnen Häutchens, welches den ganzen Embryo von allen Seiten umgab. Die beiden anderen Keimblätter beschränkten sich blos auf die Keimstreifen. Das eine von diesen Blättern, dasjenige nämlich, welches unmittelbar unter dem obersten Häutchen lag, bestand aus einer Reihe grosser Zellen, welche in vier Reihen in jedem Keimstreifen geordnet waren. Das untere, dicht dem Dotter anliegende Blatt erschien in Form eines dicken, aus kleinen Zellen bestehenden Wulstes. Bei weiterer Entwicklung, zur Zeit wann sich die beiden Keimstreifen in ein Ganzes verschmolzen haben, erfahren die Keimblätter wichtige Umänderungen wobei übrigens das oberste dünne Blatt nur eine untergeordnete Rolle spielt. Dieses behält seine ursprünglichen Eigenschaften und erweist sich bald als die Epidermis des Embryo. Das zweite Blatt, welches nunmehr aus kleineren Zellen zusammengesetzt wird, bildet dann das centrale Nervensystem."

Speaking of the germ-layers of Clepsine from a comparative stand-point, Metschnikoff remarks, — "Der Hauptunterschied bei Clepsine besteht darin, dass sich das epidermoidale Blatt sehr früh von der Nervenanlage absondert. . . . Die beiden ersten Keimblätter von Clepsineembryonen werden somit dem oberen Blatte des Skorpions und anderer Articulaten entsprechen."

Independently of Metschnikoff's paper, which had escaped my attention, I came to precisely the same conclusion (No. 1); and a little later Hoffmann (No. 6, p. 42) repeated my observations on this point, without adding to or correcting them. Other writers, with a single exception, have failed to get as near the truth as this, and their observations have tended to confusion rather than enlightenment.

Bergh is the only one among the more recent writers who has made any advancement on the observations of Metschnikoff, Rathke, and Leuckart. In the course of his extensive papers on the metamorphosis of the Gnathobdellidæ, and in several reviews, he has stated very briefly the results of studies yet to be published, as I am led to infer, on the origin of the nerve-chain. According to Bergh, the original epidermal layer is lost, and the "definitive epidermis" is then formed from the lateral portions of what I have called the neuro-nephric stratum, while the nerve-cord is formed from the median portion of the same stratum. Although "median portion" is still an indefinite

quantity, it is evident that Bergh has made a closer approximation to precision than any of his predecessors. Grube is the least definite of all; Robin (No. 5, pp. 199, 344) regarded the entire germ-bands as the central nervous system. Rathke and Leuckart traced its origin to the median portion of the bands; Metschnikoff limited it to a single stratum, and Bergh to the median portion of the same stratum.

From the following comments by Bergh it will be seen that he thought it impossible to trace the nerve-cord to special neuroblasts in Aulostoma, and a little more than impossible (!) in the case of Clepsine: —

"Whitman hat die zehn Zellen am Hinterende der Rumpfkeime gefunden, welche bei Clepsine durch ganz besondere Grösse ausgezeichnet sind; die acht derselben nennt er Neuroblasten, die zwei dagegen Mesoblasten, indem er annimmt, dass aus den ersteren nur Nervensystem, aus den anderen nur Mesoderm entstehe. Vergeblich sucht man in der genannten Arbeit ebenso wie in der Natur selbst irgend einen Beweis für diese Behauptung, die eben nur eine solche ist. Es ist bei Aulostoma *vollkommen unmöglich*, die Descendenten jeder einzelnen der erwähnten grösseren Zellen für sich zu verfolgen, und *bei Clepsine wird die Sache noch viel schwieriger*, indem die Rumpfkeime hier stark gekrümmt sind. Richtig ist es aber, wenn Whitman *ganz im Allgemeinen* die Bauchkette aus den Rumpfkeimen herleitet." (No. 9, p. 259).

In Bergh's Fig. 23*a*, Pl. XV., all the strata of the germ-bands are clearly defined; but if we compare this Fig. with Fig. 24*a* and *b*, the entire neuro-nephric stratum appears to be employed in the formation of the "definitive epidermis." On page 263 the nerve-cord is said to arise *beneath* the "Anlage der definitiven Rumpfepidermis." This statement, taken in connection with the illustrations, of Pl. XV., would lead one to suppose that the nerve-chain had its origin in the inner (mesoblastic) layer of the germ-bands. Scarcely more definite are his descriptions in the case of Nephelis (No. 13, p. 295, Pl. XIX.). All the strata are clearly represented in the figures, but the neuro-nephric stratum is marked *ep* ("definitive epidermis"), and there is not the slightest indication of any distinction between neural and epidermal elements. The origin of the nervous system from this layer is conceded in a foot-note (p. 295), in

which my interpretation of the teloblasts is briefly noticed.[1] The same fact is more definitely stated in later papers (No. 20, p. 4, and No. 21, p. 408). Bergh's interpretation of the neuro-nephric stratum, as a neuro-epidermal layer, will be considered after my own observations have been presented.

For the latest contribution on the subject we are indebted to Joseph Nusbaum (Nos. 8, 22, and 23). Nusbaum has given a detailed account of the origin of the nerve-cord from the epidermal layer, thus confirming the conclusion reached by Kowalevsky and sustaining the position taken by Balfour (No. 12). Nusbaum is more fortunate in his company than in his observations, and this is almost the only reason that compels me to burden the reader with a review of his statements. His elaborate account of the mode of origin of the nerve-system from the epidermal layer, in view of the fact that this layer has absolutely nothing whatever to do with its formation, is, to say the least, a most singular production, and one for which it would seem, at first sight, very difficult to find any very satisfactory explanation. But it undoubtedly has an explanation, and I am under the disagreeable necessity of pointing it out. Nusbaum's observations, not only on the nerve-system, but on nearly every point that he has discussed, show a most evident lack of thoroughness. He has neglected to acquaint himself with the more important facts in regard to the origin and structure of the germ-bands, and the consequence is that he has misinterpreted and blundered at nearly every step. He has just as little knowledge of the anatomy as of the embryology of Clepsine; for he starts out under the persuasion that there is only one pair of testicular sacs, (No. 22, p. 614). And yet these sacs are so large and conspicuous

[1] In justice to Bergh it should be stated that his remarks on the origin of the nerve-cord are intended only to serve as a preliminary account.

(20.) BERGH, R. S. Über die Deutung der allgemeinen Anlagen am Ei der Clepsinen. *Zool. Anz.* No. 216. 1886.

(21.) Id. — Die Entwicklungsgeschichte der Anneliden. *Kosmos.* II. p. 401 1886.

(8.) NUSBAUM, JOSEPH. Recherches sur l'Organogénèse des Hirudinées (Clepsine complanata Sav.).
Arch. Slaves de Biologie. I. fasc. 2, pp. 320–340; fasc. pp. 539–556. 1886.

(22.) Id. — Zur Entwicklungsgeschichte der Hirudineen (Clepsine).
Zool. Anz. VII., No. 181, p. 609. Nov., 1884.

(23.) Id. — Zur Entwicklungsgeschichte der Geschlechtsorgane der Hirudineen.
Zool. Anz. VIII., No. 191, p. 181. Mar., 1885.

that they can easily be seen through the body-wall with the naked eye. What a spectacle is presented when one undertakes to instruct us about the genesis of organs he has never seen! In his second contribution (No. 23, p. 182) he discovers his error, but charges it to Moquin-Tandon. Has Nusbaum never heard of Leuckart, Leydig, or Claus, that he should go back to an authority of half a century ago to find out how many testiculi Clepsine has? But this is a trivial error in comparison with inaccuracies in observation and reading, such as we shall find in his description of the origin of the nerve-chain.

Nusbaum (No. 8, p. 21) first attempts to explain why others have been less successful than himself in tracing the derivation of the nervous system. He discovers — so he affirms — that the egg-membrane is composed of two layers, the *inner* of which is provided with pores. In the course of development this porous layer is penetrated with vitelline granules, and, sometimes, with protoplasmic particles. "*D'où il résulte que cette couche peut être prise assez facilement pour l'ectoderme. Alors il doit sembler que le système nerveux se forme aux dépens du feuillet moyen*, de sa couche la plus externe, aboutissant à l'ectoderme." The invention of such a blunder is as preposterous as its commission is impossible. Furthermore, such a condition of the egg-membrane as Nusbaum has represented in Fig. 32, Pl. III. is either artificial or altogether imaginary. Had Nusbaum begun his observations on the germ-bands at an early stage of their development, it would probably never have occurred to him to suspect his predecessors of such a stupid blunder. In these early stages the egg-membrane is not in contact with the germ-bands, and I fail to see how such a strange condition of the membrane could arise.

In a brief historical review, the opinion which I formerly held (No. 1) on the origin of the nervous system is cited as something "strange enough," and as opposed to the ideas of Metschnikoff and Hoffmann. I have already made it clear that this opinion coincided with that of Metschnikoff and Hoffmann. Bergh's conclusions on this point are not even mentioned.

Nusbaum has described the development of the nervous system in his preliminary article with quite as much detail as in his final paper, and in nearly the same words. I shall, therefore, give here the original description, which runs as follows: —

"Während der Embryo noch eine ovale Form besitzt und nach der Rückenseite hin stark gebogen ist, finden wir *das dünne, einschichtige Ectoderm in dem vorderen und mittleren Theile des Embryo in der Mitte der Bauchseite verdickt;* es entsteht hier eine Schicht grösserer, zuerst runder, später in kubische übergehender Zellen. *Dies ist die Anlage des Bauchnervenstranges, die eine continuirliche Schicht mit dem Ectoderm bildet, und dicht unter der dicken derben, zweischichtigen Chorionmembran zu liegen kommt. Die Zellen dieser einschichtigen Nervensystemanlage beginnen sich in der Richtung nach innen hin zu vermehren, und verursachen somit die Verdickung derselben.* Ganz unabhängig vom Bauchnervenstrange entsteht eine ähnliche ectodermale Verdickung auf dem Kopfende des Embryo, die Anlage des Gehirnganglion bildend. . . . Die Trennung des Nervensystems vom Ectoderm findet auf folgende Weise Statt. *Von beiden Seiten der Bauchnervensystemanlage bildet sich je eine dünne ectodermale Falte nach aussen hin; die beiden Falten, sehr dicht dem Chorion anliegend, wenden sich in der Richtung nach der Mittellinie der Bauchseite des Embryo, um hier später an einander zu stossen. Es bildet sich also eine Art breiter Nervenrinne, von der Seite des Chorion geöffnet; sie ist aber fast vollständig flach und seicht, so dass ihr Boden, d. h. die zuerst gebildete Nervensystemanlage, dem Chorion nahe anliegt. Nach der Aneinanderstossung der obengenannten Falten, bildet sich eine continuirliche Ectodermschicht und der Bauchnervenstrang stellt eine Art platten und breiten Rohrs mit einem excentrischen, engen und spaltförmigen Lumen, dessen innere Wand dick ist und den eigentlichen Bauchnervenstrang vorstellt, während die äussere, dem Ectoderm zugekehrte, von einer einzigen Schicht platter Zellen gebildet ist.* Diese Spalte sieht man noch eine Zeit lang nachher; auf späteren Stadien verschwindet sie spurlos. Es geht aus diesem Entwicklungsmodus hervor, dass derselbe eine nur wenig modificirte Art der Nervensystembildung der Branchiobdella darstellt, wo sich, nach Herrn Prof. Salensky, eine grosse und tiefe Nervenrinne bildet, die sich in einem Nervenrohr schliesst" (No. 22, pp. 610–611).

How beautifully all this chimes with the idea that a neural canal, comparable with that of the vertebrates, should be expected in the annelids! How much detail in describing some-

thing so eminently satisfactory in theory, but without a particle of foundation in fact!

Among the figures given by Nusbaum (No. 8), I find only one (Fig. 33, Pl. III.) in which there is a median ventral thickening of the epidermal layer that might be mistaken for the basis of the nerve-chain. I have seen the same thing, and my first thought about it was that it represented a neural thickening. I shall show that this thickening is a glandular organ, and that the nerve-chain arises beneath it, and never has any connection with it. Nusbaum gives no figures in which such a thickening is seen at any point far behind the anterior ends of the germ-bands. In his Fig. 34, representing a transverse section of some part of the trunk of the embryo, there is not the slightest evidence of an epidermal thickening. On the contrary, the nerve-chain is here sharply marked off from the epidermis, although in contact with it. How does it happen that the nerve-chain is here farther advanced in development than at the anterior end? We ought, of course, to find the development less and less advanced as we go from the head towards the hind end. The broad neural groove ("breiter Nervenrinne") shown in his Fig. 34 is purely ideal. Both Bergh and Hoffmann agree with me in affirming that the epidermal layer is here continuous, and not interrupted, as represented by Nusbaum. The epidermis completely covers the neuro-nephric stratum at a very early stage, and even advances more rapidly than the deeper portions of the germ-bands, meeting in the median ventral line, and forming a continuous layer before their junction, as shown in Fig. 7, Pl. IV. Both the "neural groove" and the "thin ectodermal folds" are inventions, pure and simple, — products of a fertile imagination, which pays more respect to the supposed requirements of some fascinating theory than to the needs of thorough and accurate observation.

Nusbaum goes completely astray in his account of the second layer (neuro-nephric) of the germ-bands, as will be seen from the following (No. 22, p. 613): "Die acht grossen Zellen, von Whitman 'Neuroblasten' genannt, die am Hinterende des Embryo früh auftreten und als Producte des primitiven Entoderms aufzufassen sind, erleiden folgende Veränderungen. Sie unterliegen einer energischen Theilung und vermehren sich fort und fort in der Richtung von hinten nach vorn. Auf einem frühen Stadium

wo das Nervensystem vom Ectoderm sich noch nicht abgetrennt hat, findet man auf einem Querschnitt durch den hintersten Theil des Embryo zwei Reihen dieser Zellen, zu vier an jeder Seite der Bauchfläche liegend; in dem etwas mehr vorderen Theile des Embryo sind nur deren zwei jederseits, und noch näher zum Vorderende beobachtet man jederseits blos eine einzige solche Zelle im Mesoderm, nahe der Bauchseite des Embryo liegend. Die Vermehrung dieser Zellen geht bis zum vordersten Theile des Embryo vor sich, so dass zuletzt in jedem Somite des Embryo jederseits je eine einzige Zelle vorkommt. . . . Diese grossen, in jedem Leibessegmente hervortretenden Zellen beobachtete auch Whitman, und nannte sie 'Segmentzellen.' Da er sich aber die Entwicklung des Nervensystems nicht richtig vorstellte und es von den 'Neuroblasten,' d. i. den acht grossen, oben erwähnten hinteren Zellen herleitete, so bemerkte er keinen genetischen Zusammenhang zwischen letzteren und den Segmentzellen. Whitman vermuthete aber nicht unrichtig dass die Segmentzellen vielleicht an der Bildung der Geschlechtsorgane (Testiculi) Theil nehmen." (Compare, No. 8, p. 17.)

The elements of the two inner and principal strata of the germ-bands are here confounded. Nusbaum appears to be entirely ignorant of the existence of the two large mesoblasts and their relation to the germ-bands. The sexual cells ("segment cells"), which are derived from the mesoblasts, and which form a part of the third (inner) layer of the bands, are identified with the cells of the second (neuro-nephric) layer. At the hind end of the embryo, as Nusbaum affirms, are found *four* rows of these sexual cells on the ventral side of each band; farther forward only *two* rows are present; and at the anterior end there is only *one* row. Four rows reduced to one! and no explanation offered. It is quite true that there are four rows of cells in the neuro-nephric stratum of each band; but the number of these cells seen in transverse section does not diminish, but increases from behind forward.

It remains to notice the opinions of a few writers who, although they have made less extended studies on the development of the Hirudinea, or none at all, are nevertheless regarded as important authorities on the subject. The conclusion reached by Kowalevsky has had greater weight with many authors than

it is really entitled to. That it is not based upon sufficiently thorough observations is evident enough from Kowalevsky's own words. "Die Embryonen der Hirudineen," says Kowalevsky (No. 10, pp. 1–2), "erwiesen sich aber zu Querschnitten nicht ganz passend, und *es gelang mir nur mit grösster Mühe, einige feine Querschnitte anzufertigen, auf welchen ich die Scheidung in Keimblätter uud die Bildung des Nervensystems aus dem oberen Blatte sehen könnte, aber nicht den Keimstreifen,* aus dem sich lediglich die Muskeln entwickeln."

In regard to Clepsine, Kowalevsky remarks (p. 3): "Da ich aber zur Zeit der Entwickelung derselben gerade mit den Accipensern beschäftigt war, so bewahrte ich *nur mehrere Stadien in schwacher Chromsäure auf; an Querschnitten derselben könnte ich mich später nur von der Abstammung des Nervensystems vom oberen Blatte überzeugen.*"

Kowalevsky's conclusion as to the origin of the nerve-system rests, then, as he himself acknowledges, on the study of a few eggs so imperfectly preserved and prepared that it was impossible to understand the structure of the germ-bands. How Kowalevsky convinced himself, with such material as this, that the nerve-chain arises from the epidermal layer, must be left entirely to conjecture; for not a word of explanation is offered, nor a single figure given.

Semper (No. 18), in his well-known work on "*Die Verwandtschaftsbeziehungen der gegliederten Thiere*," reviews the statements of Rathke, Leuckart, Kowalevsky, and Metschnikoff on the origin of the nerve-system in the Hirudinea, and endeavors to reconcile them with his own observations on Nephelis, Nais, Chaetogaster, etc. "Nach eigenen Untersuchungen," says Semper, "kann ich für Nephelis die Angaben Rathke's bestätigen, dass die Ganglien entstehen durch Sonderung der medialen Parthien des Keimstreifens; *eine mittlere, von diesem unabhängige Ectodermverdickung tritt bei dieser Gattung so wenig, wie bei Chaetogaster ein*" (p. 246).

Semper, however, maintains that the nerve-chain has a double origin, its median portion being derived directly from the ectoderm, and the lateral portions (ganglia) from the mesodermic layer of the germ-bands. This view enables him to explain the contradictory results of different writers. Metschnikoff's statements regarding Clepsine are commented on as follows (p. 178):

"Metschnikoff's inneres Blatt des Keimstreifens allein ist das Mesoderm, dessen Betheiligung am Aufbau des Bauchmarks er nicht gekannt hat; sein äusseres Blatt des Keimstreifens gehört dem Ectoderm an, und er hat hier ganz richtig dessen Theilnahme an der Bildung des Nervensystems erkannt, dagegen seine erste Entstehung aus dem ectoderm nicht beobachtet."

According to Semper, Metschnikoff and Kowalevsky are wrong only in supposing that the *whole* of the nerve-chain arises from the ectoderm, while Rathke was equally wrong in deriving it wholly from the mesoderm. Semper (p. 295) extends the idea of a double origin of the central nervous system to all segmented animals. The median part ("centrale Ganglionzellenstrang"), which is supposed to arise as an unsegmented cord, from a thickening of the ectoderm, is regarded as homologous with the spinal cord of vertebrates; the lateral ganglia, arising in the manner described by Rathke from the already segmented mesoderm, are homologized with the spinal ganglia of vertebrates.[1]

Hatschek's remarks (Nos. 24, 25) on the origin of the nervous system in the Hirudinea are of an incidental character, and are of interest only on account of the general scheme set up for the annelids. The ventral nerve-chain, according to Hatschek, arises from three rudiments, one median and two lateral. The median rudiment is a longitudinal infolding of the ectoderm along the ventral line, and is marked with a groove like the medullary groove of vertebrates. The lateral rudiments are prolongations of a pre-oral, neural plate ("Scheitelplatte"), which had its origin in an unpaired thickening of the ectoderm.

The "neural groove," which forms the most seductive feature of Hatschek's scheme, has nothing whatever to do with the formation of the ventral nerve-chain, as has been clearly shown by Kleinenberg. In concluding his excellent review of Hatschek's observations, Kleinenberg (No. 26, p. 123,) states the case thus:

[1] In a foot-note (p. 179) Semper admits that the spinal ganglia may originate in the neural plate, as maintained by Balfour; but, in this case, he would still maintain their homology with the lateral ganglia of annelids.

(**24.**) Hatschek, B. Beiträge zur Entwickelungsgeschichte und Morphologie der Anneliden. *Sitzb. Akad. Wiss. in Wien.* LXXIV. 1876.

(**25.**) Studien über Entwicklungsgeschichte der Anneliden. *Abeiten a. a. zool. Inst. zu Wien.* I. 3. 1878.

"In ihren wesentlichen Grundlagen halte ich aber meine Ansichten von der Entstehung des Bauchmarkes, wie sie 1878 für Lumbricus und 1881 für Lopadorhynchus entwickelt wurden, aufrecht, da sie durch fortgesetzte Beobachtungen an anderen Anneliden nicht bloss bestätigt sondern auch weiter ausgebildet werden konnten. *Überall entsteht der Bauchstrang unabhängig vom Kopfganglion aus zwei seitlichen Anlagen, ohne Betheiligung einer soliden oder röhrenförmigen medianen Einstülpung des Ektoderms.*"

It is remarkable that so careful an investigator as Kleinenberg should have entirely overlooked the relation of these "*seitlichen Anlagen*" to the terminal neuroblasts.

The "neural canal" of Branchiobdella, which Salensky (No. 15) homologizes with the medullary canal of vertebrates, has also been effectually disposed of by Kleinenberg (No. 26, p. 127). It has just as little morphological significance as the "neural groove" which Nusbaum describes in Clepsine. Salensky discovered eight teloblasts in Branchiobdella, but missed their special relations and significance.

Kleinenberg's studies on Nephelis (No. 26, p. 129), which were begun some time ago, and not carried to completion, failed to give him the clue to the precise origin of the nerve-chain and its relation to the epidermal layer. The loss of the original epidermis, as stated by Rathke, and more fully described by Bergh, was also observed by Kleinenberg.

Goette (No. 27, p. 91), adheres to the belief that the germ-bands are wholly mesoblastic, and discredits the testimony of those who derive from them the ventral chain, which, as every one will now admit, is ectodermic in origin.[1]

(**26.**) KLEINENBERG, N. Die Entstehung des Annelids aus der Larve von Lopadorhynchus.
Zeitschr. f. wiss. Zool. XLIV. 1 and 2. 1886.

(**27.**) GOETTE. A. Abhandl. z. Entwicklungsgesch. der Thiere. Heft. 2. Vergleichender Theil. 1884.

[1] So strong is Goette's faith on this point that he feels inspired to prepare for his heterodox brethren a very edifying homily in the form of a lengthy foot-note, warning them of the unpleasant consequences of an "einseitige Beurtheilung," and admonishing them of the saving efficacy of the "vergleichende Methode." The counter homily needs no quill from the wing of the angel Gabriel. When facts conflict with theory we know on which side the error lies; and we have little respect for a (not *the*) "comparative method," which begins by denying well-authenticated facts.

Balfour based his account of the formation of the germ-layers in Clepsine on the observations presented in my earlier paper (No. 1), and offered one or two critical remarks that deserve notice. Referring to my statements on the origin of the nerve-chain from the neuroblasts, he says (No. 12, pp. 288–9): "Such a mode of origin for a ventral ganglionic chain is, so far as I know, without a parallel in the whole animal kingdom. . . . Till more evidence is brought forward by Whitman or some other observer in support of the view that the so-called neuroblasts have any share in forming the nervous system, they must, in my opinion, be regarded as probably forming, in conjunction with the mesoblasts, two simple mesoblastic bands. Kowalevsky has, moreover, briefly stated that he has satisfied himself that the nervous system in Clepsine originates from the epiblast, — a statement which certainly could not be brought into harmony with Whitman's account."

In reply to these objections the following considerations have been offered (No. 28, p. 392): —

"With reference to Clepsine, Kowalevsky remarks: 'I preserved only several stages in weak chromic acid, and from sections of these I could only convince myself later of the origin of the nervous system from the upper layer.' This is all he has said on this point; and I will now show that, if we do not go behind the verbal statement itself, it does not even require to be brought into harmony with my account, since it is precisely what I have claimed. The four rows of neuroblasts in each germ-band lie, at the outset, at the surface, and must therefore be considered a part of the epiblast, although a specialized part. It is simply a precocious differentiation of the edge of the epiblast, by which epidermal and neural elements become distinctly marked at an unusually early stage. In the course of the epibolic growth of the ectoderm the epidermal portion progresses somewhat more rapidly towards the lower pole than the germ-bands, and thus sweeps over the neural portion. But it seems to me plainly a matter of little importance whether the neural portion loses its surface position during the epiboly, or immediately after the conclusion of the concrescence of the

(28.) Whitman, C. O. A Rare Form of the Blastoderm of the Chick, and its Bearing on the Question of the Formation of the Vertebrate Embryo. *Quart. Journ. Mic. Sc., XXIII.* 1883.

germ-bands; and I confess that I do not see wherein this view requires 'any special support.' At the time Balfour penned the above criticism he evidently was not aware that my observations on the origin of the nervous system in Clepsine were but little more than a corroboration of those of an eminent Russian embryologist."

(*b*) *Observations on the Origin of the Nerve-chain.* — The proof that the entire ventral nerve-chain arises as two simple longitudinal rows of cells, and that each row is produced by the continued proliferation of a single cell, — the neuroblast, — is to be obtained by the study of surface-preparations in connection with sections made in the planes of the three axes. Sections show that the fourfold striated appearance of the germ-bands is due to the presence of four rows of cells beneath the epidermal stratum of each band; and surface-preparations enable us to trace these rows forward into the special organs developed from them. I have been helped towards precise results by the differential action of the preservative fluids employed, and by natural distinctions between the neural and the nephric cell-rows. These distinctions are less conspicuous in the species hitherto studied in Europe than in the American species (C. parasita), and hence have been overlooked. The nephridial rows are more granular and stain more deeply with osmic acid than the neural and lateral rows. A glance at Plates IV. and V. will show how extremely useful such distinctions have been in the analysis of the neuro-nephric stratum.

Having already briefly indicated the different layers of the germ-bands, it remains to consider more in detail the elements composing the neuro-nephric stratum. As was made clear in my former paper (No. 1), there are exactly four rows of cells in this stratum in each germ-band. The outlines of these rows can easily be seen in germ-bands hardened in situ (Fig. 1, Pl. IV.), but better in surface-preparations which have been freed from the yolk (Fig. 8, Pl. V.). They are most distinctly marked at the hind ends of the bands, but can be traced forward nearly to the cephalic lobe in many preparations. The reason for their becoming less and less sharply marked as we pass from the hind end forwards, lies in the fact that development is more and more advanced in this direction. Behind, the rows are simple (*i.e.*, each consists of a line of single cells), and

often separate from one another for a short distance in front of the teloblasts. Farther forward, each row becomes double, then triple or quadruple, and at the same time its boundary lines become less clearly defined, as shown in Figs. 8–11. Fig. 7 is a transverse section of the stage seen in Fig. 8, at a point where the bands are still separated by a narrow interval. The rows of cells are here simple, each showing a single cell in section. In another section, taken just in front of this, where the bands have already closed, three median cells (Fig. 6, *nc*) are seen in place of the two shown in Fig. 7. It is possible that there has been a duplication of cells in one of the median rows, but it is more probable that the section has passed through two more or less wedged-shaped cells belonging to the same row, but inversely placed, as shown in the left neural row of Fig. 11. Fig. 11 represents a horizontal (frontal) section near the posterior end of an embryo in which the germ-bands are fully closed. All the rows are here simple, but towards the middle of the same embryo (Fig. 10) we find the neural row doubled on one side and tripled on the other. A little in front of the middle a still more advanced condition is found (Fig. 9); for here not only have the neural cells become smaller by division, but there is a plain differentiation into median (Long. commissures + median ganglia) and lateral (lateral ganglia) portions.

A more complete and instructive picture may be obtained by mounting the embryo entire, after stripping it from the yolk. In such a preparation (Fig. 8), passing from the hind end forwards, we meet with successively higher stages in the development of the nerve-chain, beginning with two simple rows of cells (*nc*) and ending with well-defined ganglia. The neural rows are so clearly delimited against the darker, nephridial rows, and the steps in development follow in such a perfect ascending series, that every doubt about the conversion of these rows into the nerve-chain is removed.

In Figs. 15–19, representing transverse sections from the posterior end of an embryo just hatched, the neural rows have united into a median flattened cord (*nc*) which shows a differentiation into median (*lc*) and lateral portions, as in Fig. 9. In these sections, selected from a series beginning in the middle of one somite and extending to the middle of the next in front, the contrast in color between the neural and nephridial elements

is well-marked. The contrast between the nephridial (*nph*) and the lateral (*m'*) cells is equally strong, so that in this advanced stage of development it is still easy to find all the derivatives of the neuro-nephric stratum, and to connect them with the primary cell-rows shown in Fig. 6.

3. *The Larval Gland-Cells.*

Passing now to the anterior end of the same embryo we find the nerve-cord presenting the same general form, with the median and lateral parts more clearly defined (Fig. 23). The median part does not yet show the double commissures. In this section, taken in the region marked *gl* in Fig. 8, we meet with a very interesting larval organ, consisting of numerous large gland-cells, each with its own duct leading to the exterior. These massive gland-cells lie between the sub-œsophageal ganglia and the epidermis, and extend over an area of greater breadth than the ganglia. These glands arise as a pair of thickenings of the epidermal layer immediately behind the cephalic lobe, and appear as rounded prominences in quite an early stage of the germ-bands. (Fig. 1). The epidermal thickening is always clearly distinct from the neural cells, as may be seen to best advantage in longitudinal sections (Figs. 20 and 21). It is undoubtedly this thickening which Nusbaum has figured and described as the basis of the nerve-chain. Only the deeper cells of the thickening are destined to become gland-cells, and these appear to sink gradually beneath the surface, the ducts forming in situ rather than by subsequent outgrowths. A median sagittal section of this stage (Fig. 28) often shows only a few gland-cells compared to the number met with in sections passing about midway between the median ventral line and the side of the embryo (Fig. 29), showing that they still form two more or less distinct groups.

Nusbaum (No. 8, p. 28) has described a "provisional dorsal organ" entirely different from anything I have seen. "Chez l'embryon, dont le système nerveux est déjà complètement séparé de l'ectoderme, j'ai remarqué, au milieu de la paroi dorsale du corps, dans la troisième partie antérieure de sa longueur, une couche de cellules hautes, cylindriques, de l'ectoderme. . . . Les cellules ectodermiques, qui forment cette proéminence, emettent ensuite des fils externes, minces, très

longs. . . . Le rôle physiologique de ces fils consiste, comme il me semble, dans la fixation réciproque de jeunes individus, tournés ordinairement l'un vers l'autre par leurs faces dorsales et fixés à la paroi ventrale de la mère par leurs ventouses antérieures. Cet organe dorsal n'existe cependant pas longtemps; il disparaît sans laisser de traces avant la séparation des jeunes individus de la paroi du corps maternel."

Nusbaum and Hoffmann (No. 4, p. 45) have both fallen into the same error of supposing that the larvæ attach themselves to the ventral side of the parent by the oral sucker. The young are further, according to Nusbaum, fixed to one another by means of glutinous threads formed by the "dorsal organ."

While studying the embryology of C. marginata and complanata, I noticed that larvæ hatched from eggs that were taken away from the parent and kept in a watch-glass, soon became attached to one another in pairs. The point of attachment, however, was not dorsal, but ventral, just behind the part destined to form the oral sucker. The attachment, as I have since learned, is effected through an adhesive secretion of the larval glands above described. When the leech is allowed to remain over the eggs until they hatch, the larvæ become fixed to its underside, not by the still undeveloped oral sucker, but by the secretion of the post-oral, ventral glands. I have never noticed the "reciprocal attachment" by means of a "dorsal organ;" but, without further examination, I would not venture to dispute Nusbaum's statement. But the description and figures given certainly awaken the suspicion that the "dorsal organ" is a pathological formation. The larval glands which I have described serve only the temporary purpose of fixing the young to the parent leech at a time when neither sucker is sufficiently developed to perform this office. As soon as the posterior sucker becomes serviceable, it is used as an organ of attachment, and the larval glands disappear; at least, I have not been able to connect them with any organ in the adult leech.

The larval organs of adhesion occupy a position which corresponds to that of the Ganoid suctorial disc. The means of fixation in the young fish (Amia), at least in the youngest larvæ, is an adhesive secretion. My attention was first called to the secretion in Amia by Dr. Patten.

4. *Primary Sense-organs of the Lip.*

At the time of hatching, long before the eyes and their serial homologues, the segmental sense-organs, appear, two pairs of sense-bulbs are found, symmetrically placed on the surface that is to form the margin of the lip. These organs arise as bulb-like thickenings of the epidermis. Figs. 12 and 13 represent successive sections through the anterior pair of bulbs (*sb'*), in the direction shown by the arrows 12–12 and 13–13 in Fig. 29. The fifth section behind that seen in Fig. 13 hits the posterior pair of bulbs (*sb''*), passing above the super-œsophageal ganglia, as indicated by the arrow 14–14 in Fig. 29. These sense-bulbs are nearer together than those of the anterior pair, and they are a little depressed, as if there were a slight infolding. The scanty material at my disposal did not permit me to trace the history of these organs farther. I have since given some time to the study of the development of the eyes and sense-organs of the lip, in much later stages, and I have found that all the sense-organs of Clepsine arise in the same manner as these two pairs of bulbs. As the entire œsophageal nerve-collar is already formed, there is absolutely no ground for supposing that these bulbs are rudiments of the nervous system. The basis for the super-œsophageal ganglia (*sup. æ. g.*) is present as a distinct body of cells in the stage of Fig. 20, long before the appearance of the primary sense-bulbs. Even as early as the stage of Fig. 3, I find between the epidermal layer of the cephalic lobe and the primary entoderm cells a layer of cells which I regard as the basis of both the neural and the mesodermic elements of the head. The precise origin of this layer I have not thus far determined.

5. *The Nephridia.*

In describing the origin of the nerve-chain I have called attention to the nephridial rows of cells, two of which are found in each germ-band, lying between the median (neural) and the lateral rows (Figs. 2, 6, 7, 8, 9, 11, *nph*), and forming one stratum with them. These relations are shown in Diag. 9.

In C. parasita the nephridial rows are remarkably distinct, owing to the contrast in color between them and the rows by which they are bounded. This contrast, due to the coarse

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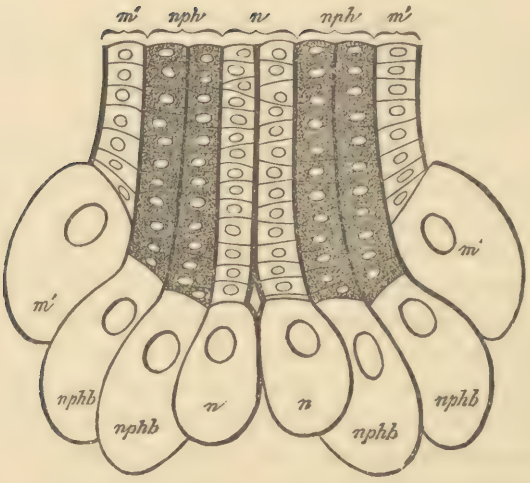

Diag. 9. — *A diagrammatic surface-view of the neuro-nephric stratum at the posterior end of the nearly completed germ-bands.*

n, neural rows; *nb*, neuroblasts; *nph*, nephridial rows; *nphb*, nephroblasts; *m'*, lateral rows.

granules of the cells, is strengthened by the action of osmic acid, and thus becomes a most important aid in determining the fate of the cell-rows.

Tracing the nephridial rows forward in a surface-preparation (Fig. 8, Pl. V.), we find each represented behind by a line of single cells; towards the middle, they become double or triple, while still maintaining the same diameter; near the beginning of the anterior third, the two rows blend; and here outlines appear, at first shadowy, then more distinct as we advance, cutting the rows into quadrangular plates with rounded angles. The formation of the nephric plates progresses from the cephalic end backwards, keeping exact pace with the metameric division of the embryo. Thus the basis is laid for a pair of nephridia in each somite, although only sixteen pairs are retained in the adult. The details of the process by which these plates are converted into the nephridial organs I have not attempted to follow. It is a point, however, which is worthy of a most careful study.

The series of sections shown in Figs. 15–19, beginning near the middle of a posterior somite, and running forwards to the middle of the next in advance, shows that the cells of the nephric plates multiply in depth as well as breadth. Fig. 18 represents a section on the boundary-line of two somites, in which not a single nephridial cell could be found. It is in this region that we meet with a pair of large cells (*sex*) lodged in the mesoderm. A single pair of these cells occurs in each somite, and their position in the walls of the septa suggests that they may be the mother-cells of the testicular organs. Nusbaum claims to have traced the development of these cells into the sexual organs; but he has evidently confounded the cells of the neuro-nephric stratum with the sexual cells.

Returning to the nephridia, the points of chief interest in their development appear to be the following: —

1. Derivation from the ectoderm.

2. Earliest appearance in the form of simple, longitudinal cell-strings.

3. Each nephridial cell-string is a product of a single terminal cell, — the nephroblast.

As soon as I became aware of the precise origin of the nephridia, I began to question the validity of the opinion that the nephroblasts were ectoblastic. It was almost universally believed that the nephridia take their origin in mesoblastic elements. In view of this, I did not venture to discuss the question in my preliminary paper (No. 16).

Wilson's paper on Lumbricus settles the point, leaving little room for a reasonable doubt as to the ectoblastic nature of all the teloblasts concerned in the production of the neuro-nephric stratum. The establishment of this fact, taken in connection with recent discoveries pointing to the ectoblastic origin of the vertebrate segmental ducts, paves the way to a better understanding of the phylogenetic derivation of these organs.

In this connection Bergh's discovery that the larval nephridia of the Gnathobdellidæ arise as lateral outgrowths from the germ-bands is especially important. Adding this to the discovery of distinct nephridial cell-strings, we have a remarkably perfect picture of the more important steps in the development of the pronephros of Petromyzon.

6. *The "Pharyngeal Clefts."*

In Fig. 1, Pl. IV., are shown two remarkable grooves just in front of the thickened anterior ends of the germ-bands, marking the line of junction with the cephalic lobe. These groove-like formations are remarkably distinct in C. complanata, and are found in every species of Clepsine that I have examined. I have before described them as "pharyngeal clefts" (No. 1, pp. 60–61), but I now have considerable doubt as to the correctness of this interpretation. They were not so well marked in C. parasita, and I have not thus far obtained the material necessary for a detailed study of them.

Salensky (No. 15, p. 25) describes in Branchiobdella what he calls a "bifurcation de la gouttière médullaire," which corresponds nearly in position and appearance with the "pharyngeal clefts" of Clepsine.

IV. SPECIAL AND GENERAL QUESTIONS.

1. *Larval Nephridia.*

No larval nephridia have thus far been discovered in the Rhynchobdellidæ, and the relations of these organs to the permanent nephridia in the Gnathobdellidæ have not yet been made clear. Bergh was not aware of the existence of distinct nephric cell-strings, and hence his descriptions and figures do not settle the place of origin of the larval nephridia with the precision that could be desired. It would seem from Bergh's statements that these organs are derived from the two "outer strings," *i.e.*, the lateral row (*m'*), and the adjacent nephridial row in my figures. "An den äusseren Strängen sind als schräg nach aussen und hinten gerichtete Zweige die Anlagen der Urnieren, deren Aulastoma vier Paare besitzt, sichtbar. . . . Die vier Urnierenpaare entstehen somit als seitliche Sprossen von zwei Längssträngen, welche letztere sich später mit den erwähnten inneren Strängen vereinigen" (No. 29, p. 91). As the nature and fate of these "longitudinal strings" remained

(29.) BERGH, R. S. Thatsachen aus der Entwicklungsgeschichte der Blutegel. *Zool. Anz.* VII. No. 160, p. 90. Feb. 18, 1884.

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(OVER)

unknown, Bergh (No. 30, p. 115) was led to believe that "die Segmentalorgane typisch segmental, *ohne die geringste Verbindung untereinander entstehen.*" In regard to the relations of the larval to the permanent nephridia, Bergh says: "Bei den Blutegeln tritt mit grosser Klarheit hervor, dass *Urnieren und Segmentalorgane durchaus nichts miteinander zu thun haben.* Letztere legen sich nämlich (in den Rumpfkeimen) erst an nachdem die Urnieren sich schon lange von diesen abgelöst haben. Ebensowenig kann von einem ursprünglichen Zusammenhang zwischen den einzelnen Anlagen der Segmentalorgane die Rede sein (No. 30, p. 113).

If we examine Bergh's Fig. 5, Pl. XII. (No. 9), in the light of what we now know about the origin of the nephridia, we see at once that the provisional nephridia arise from one or both of the cell-rows, which must be identified with the nephridial rows of Clepsine. In this figure, the fourth nephridium ("Urniere") of the left side is represented by a single cell, which still retains its original position in the outer nephridial row. It is thus highly probable, if not quite certain, that both the larval and the permanent organs do arise from the same basis, — the nephridial rows. Allowing this to be the case the relations of the two sets of organs would be very clear. One thing is perfectly certain: it can no longer be said with Bergh and Vejdovsky (No. 31, p. 123), that the permanent nephridia arise from disconnected bases or rudiments.

The view first advanced by Hatschek (No. 25), in spite of the theoretical objections raised by Balfour (No. 32, pp. 565–6) and the lack of confirmation on the part of other writers, is, after all, the one most easily reconciled with the results presented in this paper. In my opinion it not only accords better with known facts, but presents a more rational basis for explaining the morphogenetic relations of these organs, than the theory of disconnected rudiments. I refer, of course, not to the details of the conditions described in Polygordius, but to general features; such as, the derivation of the whole excretory system of the head and trunk from a common basis, and the formation of the trunk

(30.) Bergh, R. S. Die Exkretionsorgane der Würmer. *Kosmos.* II. p. 97. 1885.

(31.) Vejdovsky, F. System und Morphologie der Oligochaeten. Prag, 1884.

nephridia by metameric division of a pair of continuous longitudinal rudiments. There can be no doubt about the homology of the nephridial rows in Clepsine and Lumbricus with the longitudinal "cell-strings" of Criodrilus; and the ciliated posterior ducts, which appear to develop as sprouts from the "head-kidneys" in Polygordius, are only more highly developed forms of the simple nephridial rows I have described.

Nephroblasts were not discovered in Criodrilus, but we may now be almost certain that they are present. We may be equally confident, I think, that nephroblasts, or equivalents, are present in Polygordius. Until they are discovered and their exact relations to the "head-kidneys" made out, it will be difficult to decide the question as to the strict identity of the larval nephridia in the Gnathobdellidæ with the cephalic nephridia of marine annelids, such as Polygordius, Echiurus, Eupomatus (Serpula), etc.

Balfour (No. 32, p. 567) was very decided in the opinion that "the provisional excretory organs of the leeches cannot be identified with the anterior provisional organs of Polygordius and Echiurus." The question now stands in a somewhat different light. Vejdovsky (No. 31, pp. 121–122) has discovered larval excretory organs in Rhynchelmis, Aeolosoma, Nais, and Chætogaster, and thinks it probable that they occur in the early embryonic stages of all the Oligochæta. Bergh has traced the development of such organs in the leeches; and his observations, in connection with mine, make it almost certain that both the provisional and the permanent organs arise from the same cell-cords. Wilson has made the important discovery of nephric cell-cords in Lumbricus; and this, in connection with the wide-spread occurrence of teloblasts, leaves little room to doubt that such cell-cords are common to all annelids. The case is made still stronger by the earlier observations of Hatschek on Polygordius, Echiurus, and Criodrilus, and by E. Meyer's discovery (No. 33, p. 677) of a longitudinal canal connecting the permanent nephridia of Terebella (Lanice) conchilega. The general occurrence of these larval organs, their relatively early origin

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(32.) BALFOUR, F. M. Comparative Embryology. II. 1881.

(33.) LANG, ARNOLD. Die Polycladen. *Fauna und Flora des Golfes von Neapel. Monographie* XI. 1884.

from, or in connection with, the nephric cell-cords, the general uniformity in their position, their non-metameric character, their atrophy and replacement by the permanent, metameric nephridia, appear to indicate that they all belong to one and the same system of organs. So far I am in accord with Bergh (No. 9, pp. 269–272, and No. 30, p. 116); but I am not of his opinion that this conclusion makes it impossible to homologize the larval with the permanent organs.

2. *Significance of Nephric Cell-Cords.*

The important bearing of the discovery of nephric cell-cords on the question of the derivation of the vertebrate nephric system has been ably presented by Wilson. Without entering into the discussion of this side of the question, I may say that I fully concur in his general views on this subject. There is one point only to which I will briefly call attention. If both the provisional and the permanent nephridia arise from the same cell-cords, how are we to know, from the occurrence of such cords, which system, if either, has been retained in the vertebrates? We may, as it seems to me, be quite certain about the homology of the nephric cell-cords, and yet be quite unable to decide whether one, both, or neither of the two nephridial systems seen in the annelids is represented in the vertebrates. We are not even certain that the larval nephridia represent the same system throughout the annelids. The mode of origin of the larval organs in leeches, as lateral-buds, reminds one of the formation of the pronephros in Petromyzon; but the outgrowths[1] from the "segmental duct" have a metameric arrangement. In the formation of the permanent nephridia of leeches we have the metameric arrangement without the lateral outgrowths, the entire cell-cords being cut up into consecutive cell-plates. The fundamental importance of homologous nephric cell-cords is not, however, lessened by any such difficulties in identification as are here presented.

[1] Scott (Morph. Jahrb. VII.) states that the pronephric funnels arise as outgrowths from the segmental duct, while Shipley (Quart. Jour. Mic. Sc. XXVII., Jan., 1887, p. 344) represents them as arising from a groove in the parietal peritoneum. As this groove (which is continuous with the lumen of the segmental duct) closes up, it leaves four or five openings which persist as the openings of the ciliated funnels.

3. *Questions Relating to the Nephridia.*

A few questions of a general nature remain to be considered. What morphological element (or elements) represents the primitive nephric basis? Is the non-metameric (larval) system to be regarded as the main stock from which the metameric (permanent) system arises by a process of budding, as held by Hatschek? Or are the relations of the two systems better expressed when both are represented as buds from a common stock? In either case what is the primitive form of the stock itself? Is it a pair of simple cell-cords, or a pair of single cells? What was the original function of these organs?

The answers to these questions will vary according to the views we entertain on the origin and significance of the metamere and its genetic relations to the head. This problem logically takes precedence of the others; but we are not yet in a position to solve it, and a presentation of the leading theories could not well be brought within the limits of this paper. Besides, such work is rendered unnecessary by the excellent review given by Fraipont (No. 34, pp. 102–125) in the latest of the Naples Monographs. Bergh has recently given a comprehensive and critical review of all that is known in the comparative morphology of the excretory organs of the Vermes (No. 30 and No. 21, p. 417). I shall therefore limit myself here to a few suggestions, which appear to be warranted by the facts presented in this paper, when considered in the light of what was previously known on the same subject.

Original Function. — Of the two functions now served by the nephridia, which is primary and which secondary? Bergh (No. 30, p. 120) holds that "*die segmentierte Leibeshöhle der Anneliden den Höhlen der Geschlechtsfollikel der Plattwürmer und Nemertinen homolog ist; jede Hälfte einer Segmenthöhle mit dem sie begrenzenden Epithel entspricht einem Geschlechtsfollikel.* Um diesen Vergleich durchzuführen, muss man sich vor allem das Verschwinden des Parenchyms bei den Anneliden vergegenwärtigen. Dabei legen sich die Wände benachbarter Follikel (Mesodermsegmente) aneinander und in dieser Weise

(35.) FRAIPONT, JULIEN. Polygordius. *Fauna u. Flora des Golfes von Neapel. Monographie* XIV. 1887.

entstehen einerseits die Mesenterien, anderseits die Dissepimente."

In harmony herewith, it is inferred that the permanent nephridia served primarily as ducts for the escape of the sexual products, the excretory function having developed later. This view may appear plausible enough so long as we assume with Bergh that the larval and the permanent nephridia represent two unrelated systems of organs, having no connection with each other either ontogenetically or phylogenetically. But, let their homogeny be conceded, and there will be no escape from the conclusion that the functional relations of the permanent nephridia to the sexual organs, wherever such relations exist, have been acquired secondarily. The question then becomes simplified; for we have only to determine the primitive function of the provisional nephridia. As these organs never function as sexual ducts we have no reason to suppose that they have ever served any other purpose than that of excretory organs. As the permanent nephridia arose later, either directly from the larval organs, or, at least, from the same basis; as they exhibit the same general structural features; and as their appearance is followed by the atrophy of the larval system, there is every reason to believe that they assumed the work of the organs which they superseded. It is easy to understand how such organs could be pressed into the service of the sexual organs, and how their original function might be suppressed as the result of adaptation to this new work. The conversion of sexual ducts into excretory ones, presenting the typical structure of the primordial excretory organs, could not, on the other hand, be so readily explained.

Original Basis. — The question of original basis, like that of original function, must be considered in the light of what is known about the development of the larval nephridia. In the leeches these organs appear to arise from single cells, which develop, by division, into simple cell-cords. This simple mode of development is repeated in the ontogeny of the metameric nephridia, as seen in the formation of nephridial cell-rows from terminal nephroblasts. Although the nephric cell-plates, into which the primary cell-cords are metamerically divided, consist of numerous cells, it is probable that each plate represents a simple (or double) string of cells, with its

coils so closely packed that the linear arrangement of the cells is obscured. I think this is a fair inference from the appearance of the nephric plate. (*Vide* No. 1, Fig. 92, Pl. XV.)

The larval nephridia of other annelids, so far as known, consist at most of only a few cells; and in some cases, *e.g.*, Eupomatus, the duct of the fully developed organ is formed within a single elongated cell, stretching from the œsophagus back to the mesoblast of the same side. A number of spherical cells are found around the anterior end of this elongated cell, and these are regarded by Hatschek (No. 35, p. 143) as belonging to the excretory organ. *The entire organ arises from two cells*, one of which forms the duct, while the other splits up into the spherical "end-cells" (p. 134). Whether the two cells arise by successive divisions of the mesoblast, or by division of a primary nephroblast, we are not informed by Hatschek. Both cells are regarded as mesoblastic, but this interpretation would be perfectly consistent with the second mode of origin.

Hatschek finds a pair of primary mesoblasts ("Urmesodermzellen"). Each of these divides into two unequal parts, a large "pole-cell" and a small "daughter-cell." The "pole-cells" evidently correspond to the two "mesoblasts" of Clepsine; and the "daughter-cells" appear to me to represent nephroblasts. But, if Hatschek is right in regard to the origin of these cells, there is one difficulty in the way of identifying the "daughter-cells" with the nephroblasts; for the former are mesoblastic, while the latter are ectoblastic. If, however, we examine the facts a little more closely, the objection appears less formidable than at first sight. In Clepsine we have seen one cell give rise by division to the mesoblasts, the nephroblasts, and the neuroblasts. The first division separates the cell into a "primary mesoblast" and a "neuro-nephroblast." The point of fundamental importance for our comparison is the *twin origin* of these cells. If we call one cell mesoblastic and the other ectoblastic, that is a matter of interpretation, which may be justified by appearances in the one case, and contradicted by them in the other. The fact remains, that the genetic relation between mesoblast and nephroblast is equally close in both

(35.) Hatschek, B. Entwicklung der Trochophora von Eupomatus uncinatus Philippi (Serpula uncinata). *Arbeit. a. d. zool. Inst. z. Wien. VI.* H. 1., p. 121. 1885.

cases; and no artificial lines of distinction, such as we are accustomed to draw between the germ-layers, can lessen its significance. When our definitions of the germ-layers fail us we must appeal to *the precise genealogy of the cells*. To deny the existence of a mesoderm is of no avail; for, with two primary layers, — ectoderm and entoderm, — we are just as far from being able to settle the question of morphological identity. When, as in the case under consideration, we find an organ arising sometimes from the ectoderm, and at other times from the mesoderm, we have to admit that there is no fixed and impassable boundary-line between these two layers; and that its association with this or that germ-layer is not an infallible guide to its morphological identity.

The following view offers a fair explanation of the point in question: Both the mesoblasts and the nephroblasts arose primarily from a common ectodermic basis. The genetic relations of the two cells have remained essentially the same; but the time of their differentiation as distinct cells varies. If the division takes place within the ectoderm, then each makes its exit from the original seat separately and independently of the other; if, on the other hand, the division is delayed until after the separation from the ectoderm is accomplished, then the nephroblast appears to arise from the same source as the mesoblastic bands, and thus to form a part of these bands. The differences noted between Eupomatus and Clepsine may be reconciled in this way. The conflicting accounts given of the origin of the vertebrate "segmental duct" admit of a similar explanation.

There are, then, some very positive indications that the larval nephridium consisted, originally, of a single cell; and the general occurrence of nephroblasts, as the basis of both systems of organs, is in favor of this view.[1]

4. *The Origin of the Epidermis.*

Bergh has shown that it is necessary to distinguish between the larval and the definitive epidermis of the Gnathobdellidæ.

[1] I am reminded of the opinion long ago expressed by Leuckart (No. 17, pp. 698–699), that the teloblasts of Clepsine ("Colossale Zellen") represent "*Urnieren*." Leuckart supposed, however, that they were provided with ducts, and that they were functionally active.

The larval epidermis arises in precisely the same manner as the epidermis of Clepsine; it is lost during larval life, and replaced by the "definitive" epidermis, which is an entirely separate and independent formation from the neuro-nephric stratum, having absolutely no direct genetic relations with the original epidermis. The origin of the definitive epidermis, as described by Bergh, has no parallel in other animals, and it is clearly impossible to reconcile it with my observations on the fate of the neuro-nephric stratum. The mode of reconciliation suggested by Bergh (No. 20, p. 6), according to which the epidermis of Clepsine is the homologue, not of the larval, but of the definitive epidermis of the Gnathobdellidæ, must be set aside as entirely incompatible with the facts presented in this paper.

There can be no doubt about the accuracy of Bergh's observations on the loss of the larval epidermis; but his theory of the origin of the definitive epidermis I am not able to accept on the evidence adduced. I have shown that three of the four rows of cells constituting the neuro-nephric stratum of each band are employed in the formation of the nerve-chain and the nephridia. I have not been able to satisfy myself fully as to the fate of the fourth or lateral row; but I have followed it far enough to ascertain that it has nothing whatever to do with the formation of the epidermis. The epidermis is perfectly distinct at every stage from the neuro-nephric stratum, and I cannot discover the shadow of a reason for thinking that it ever receives any contributions from this stratum.

In this connection I must mention one fact which links the teloblasts with the epidermis. I have shown that the neuroblast is the twin cell of the median nephroblast. The mother-cell (No. 1, Pl. XII., Fig. 35, x^3), before dividing into these two cells, produces a small median cell (x^4), which, together with its homotype of the opposite side, is converted directly into true epidermal cells. This pair of epidermal cells (x^4) is a constant and striking feature of the stage referred to. This is the nearest point of connection between the epidermic layer and the neuro-nephric stratum. But I venture to say that no one acquainted with the development of Clepsine would risk the suggestion that the whole epidermis is derived from this median pair of cells.

Kleinenberg (No. 26, p. 129) confirms Bergh's statement as to the loss of the original epidermis in Nephelis, but raises objec-

tions to his idea of the origin of the definitive epidermis. Kleinenberg adds (p. 130), that in all Polychæta, whose development is known to him, the epidermis of the larva is replaced by the definitive, outer epithelium of the annelid; but this takes place through a process of transformation, which has its point of departure in the larval epidermis itself, and only in a few cases are the parts of the old ectoderm actually thrown off.

Is it not possible that the permanent epidermis in Aulostoma and Nephelis has its origin in the larval epidermis? Such an origin would accord perfectly with what takes place in other annelids, and remove the apparent discrepancies in development between the Rhynchobdellidæ and the Gnathobdellidæ. Bergh's figures appear to lend no support to the suggestion; but important points in the history of this neuro-nephric stratum have escaped his attention, and a reëxamination is required in order to settle them. If it turns out that this stratum gives origin to the nephridia and nerve-chain, as in Clepsine, the conformity in development between the two classes of leeches will be settled beyond a doubt.

5. *Significance of the Teloblasts.*

The teloblasts form one of the most remarkable features of annelid development. They represent specialized centres of proliferation, with most marvellous powers of assimilation and reproduction. Their occurrence in worms, molluscs, and vertebrates (only mesoblastic teloblasts have thus far been discovered outside the annelids), in larval as well as in fœtal types of development, makes it sufficiently evident that they are not to be regarded as an accidental phenomenon without morphological significance.

The embryos of all bilateral animals, from the worms up to the vertebrates, lengthen by cell-proliferation at the posterior end. The question arises, Is this proliferating power invariably localized in special cells or groups of cells? It is generally believed that the posterior end of the embryo represents a mass of indifferent, non-specialized elements. It is supposed that here histological differentiation has its vanishing point; that here the germ-layers blend in a common basis.

The case of Lumbricus (*vide* Wilson's paper) shows us that

the teloblasts may differ so little in size from the cells which they produce that their terminal position is about the only means of distinguishing them. This accounts for their having been overlooked by such embryologists as Kowalevsky, Kleinenberg, and Hatschek. In the Hirudinea we see the different kinds of teloblasts of each band represented either by one or two cells. Wilson informs me that in one species of Lumbricus he finds only one nephroblast on each side; in another species, two, as in Clepsine.

We do not know to what extent this variation in number may be carried; but it adds another difficulty of recognition, which might easily become insuperable. Instead of only one or two teloblasts of a given kind, there may be many, all taking equal shares in a common work, or correlative parts of a complex work. Some such condition may be supposed to exist in the higher bilateral animals.

We already have sufficient grounds for regarding the teloblasts as an archaic feature of development. Obviously they do not represent primitive organs, but the undeveloped, embryological bases of such organs. They constitute the trunk-bud, and are thus the primary seat of all the truly metameric elements of the animal. Primarily they represented, as we have reason to suppose, the bases of non-metameric organs, in which the regenerative power was, or became, preëminent.

6. *The Fœtal and the Larval Type of Development.*

The relations of the fœtal and the larval types of development have never been made clear by those who hold that the latter represents, approximately, the ancestral line of development. Some have maintained that the phylogenetic history of the annelid is retraced in larval metamorphoses; while others have denied any such morphogenetic significance to the larva, claiming that it is a secondary form reached through adaptive changes which have been called forth by its pelagic mode of life.

Balfour has given us a broad and comprehensive discussion of the nature, origin, and affinities of larval forms, and has considered, in a general way, the nature and extent of the secondary changes likely to occur in the fœtal or the larval state. According to Balfour (No. 32, p. 299), "the relative chances

of the ancestral history being preserved in the fœtus or the larva may be summed up in the following way: There is a greater chance of the ancestral history being *lost* in forms which develop in the egg; and of its being *masked* in those which are hatched as larvæ."

Balfour's phylogenetic conclusions were based on a comparison of the various larval forms with one another. No attempt was made to identify larval with fœtal features of development, and to verify in this way deductions based on the occurrence of similar larvæ in different groups. It cannot be denied, however, that in this direction lies a crucial test of our theories respecting larval forms. As long as it remains impossible to find a parallel in fundamental features between the fœtus and the larva, so long will it be impossible to decide how much is ancestral and how much adaptive in the larva.

In spite of volumes devoted to the discussion of the subject the larva of Polygordius still remains a morphological puzzle. After an extended, critical analysis of the leading theories relating to this larva, Fraipont closes his magnificent monograph on Polygordius with the following confession: "It is not yet possible, in the present state of our knowledge, to determine what is the morphological significance of the larva of Polygordius, the Trochophora (Trochosphere) of the annelids."

In the history of the teloblasts we find a satisfactory basis for the direct comparison of the fœtal with the larval course of development. What, then, is the fœtal Trochosphere? and of what importance is it to our theoretical conceptions of the annelid embryo? Does it throw any light on the structure of the ancestral Trochosphere, — the Trochozoon? and does it assist us to a better understanding of the nature and extent of the abbreviations and modifications represented in direct development?

For the purpose I have in view Clepsine furnishes an excellent example of the direct type of annelid development, while Polygordius affords a well-known example of the larval type. These forms may, therefore, serve as points of departure for the few suggestions to be offered here. The development of the Trochosphere of Polygordius is very imperfectly known, but the gap is now bridged by Hatschek's studies on Eupomatus (No. 35).

We have seen that radial symmetry, so far as outward appearances go, is preserved in the egg of Clepsine until the eight-cell stage is reached; and that bilateral symmetry attains its fullest expression through the cleavage of the posterior macromere, which ends in the establishment of ten teloblasts. In Eupomatus we reach the same important stage of development by the time the blastopore has been reduced, by closure advancing from behind forward, to a small pore, — the future mouth. The radial Gastrula has passed into the bilateral, embryonic stage of the larva. The teloblasts are represented by a pair of mesoblasts ("pole-cells") and a pair of nephroblasts. The neuroblasts are not distinguishable, which may be explained on the supposition that they still lie in the ectoderm, and present no conspicuous differential characters. A præ-oral band of cilia (prototroch, Kleinenberg), occupying an equatorial position with respect to the primary axis, is already present, and the apex of the præoral lobe (Scheitelfeld) bears a few long cilia. With the exception of the ring and the tuft of cilia, the organs of the larva are as yet undeveloped, and exist only in the form of more or less definite rudiments. The *embryonic Trochosphere*, as we may call this stage, is represented in Hatschek's Figs. 25 and 26, Pl. XI.

In Clepsine, as I have said, the formation of the teloblasts, as the closing act of cleavage, brings us to the stage which corresponds most nearly to the embryonic Trochosphere. For the sake of distinction this stage (Diag. 4) may be named the *fœtal Trochosphere*.

In both forms we meet with the same fundamental features, and the differences are precisely such as general principles would lead us to anticipate. Ciliated organs of locomotion, specially adapted to the needs of a roving, larval life, but without functional importance for the fœtus, are not developed in Clepsine. As the fœtal Trochosphere is supplied with a large stock of food-material, ready for absorption without the aid of a digestive system, there is no necessity for an early development of the mouth and gastric cavity, such as must exist in the case of the embryonic Trochosphere; and, accordingly, we find the larval development far in advance of the fœtal in these particulars. In respect to the trunk-bud (teloblasts), the case is reversed; for in the larval embryo the differentiation of the bud

is incomplete, and its development is retarded in the interest of the Trochosphere proper; while in the fœtal form, the trochospheral development is abbreviated for the sake of a more perfect bud with accelerated development.

Among the more important differences remaining to be noticed are those which have been brought about under the influence of the food-yolk. The process of gastrulation, the form of the blastopore and its relations to the mouth, have been very profoundly modified in this way. The trunk-bud of the fœtal Trochosphere has been carried far from its original, post-oral position; and, as the result of this displacement, we see the halves (germ-bands) of the trunk, which develop side by side as a unit in the larva, formed separately, and carried over the massive sphere of yolk in such a manner as to meet along the median ventral line. This whole process of circumcrescence and concrescence has arisen secondarily, in adaptation to fœtal conditions that do not exist in the larval form. The blastopore, if we include the space traversed in the closure of the germ-bands, has been stretched out of all proportion to its original dimensions, so that it no longer represents the primitive Gastrula-mouth, but merely a secondary prolongation of it backwards along the whole ventral line of the body. In the embryonic Trochosphere we find the blastopore already closed before the trunk-bud begins to develop; hence the line of closure ("Gastrula-raphe") is limited to the ventral line of the Trochosphere. As the metameric body-region is not yet developed, it is evident that the posterior limit of the primitive blastopore falls within the non-metameric region, from which the head-segment of the adult animal is formed. This region is represented in Clepsine by the cephalic lobe, and the primitive blastopore is not, strictly speaking, represented at all. The most that can be said is that its position is sometimes indicated by a linear depression (No. 1, p. 55), extending from the mouth to the hind edge of the cephalic lobe. The line of junction of the germ-bands becomes continuous with the post-oral groove; but the two things have nothing further in common, and they are as distinct in meaning as in mode of origin. What is usually called the blastopore is therefore a secondary opening resulting from the mechanical separation of the halves of the trunk-bud, and its closure is simply a restoration of original conditions.

This closure advances from before, backwards, following the direction of the bud-development. The closure of the primitive blastopore, on the contrary, progresses in the opposite direction, and represents, not a restoration, but a reconstruction. In the embryonic Trochosphere the anterior remnant of the blastopore persists as the mouth, while in the fœtal Trochosphere, where the primitive blastopore never comes to development, the mouth appears to form as a secondary perforation.

Grant that teloblasts exist in both the larval and the fœtal form, and that the conception of them as a trunk-bud is correct, and I see no escape from the above view of the blastopore. The larval form has the primitive blastopore with typical relations to the mouth and to the non-metameric portion of the animal; the fœtal form has lost the primitive blastopore, and acquired a secondary one, which may be regarded as a posterior extension of the original opening along an entirely new region — the metameric trunk-region.

The primitive Gastrula stage is passed long before the establishment of the first metamere; the secondary Gastrula is the primitive one extended, and so retarded in development that the process of gastrulation is prolonged through the whole formative period of the embryo.

The occurrence of the Trochosphere, its origin from a typical invaginate Gastrula, the persistent relations of the blastopore to the mouth, and the presence of a teloblastic trunk-bud, all appear to me to support the views developed in the foregoing comparison.

It is conceivable that the Gastrula from which the Trochosphere arises represents not a primitive, but a derived form, which has been much reduced in extent through a retarded development of the trunk. The objections to this view are numerous, and so obvious that they need not be enumerated.

The comparison above made between the fœtal and the larval Trochosphere has important bearings on the interpretation of the blastopore in higher forms, on the concrescence theory of the formation of the vertebrate embryo, and on some recent theories of the origin of metameric segmentation, — bearings which cannot be considered *in extenso* within the limits of the present paper. I may say, however, that I see no reason for abandoning the so-called theory of concrescent growth. In all

cases where separate germ-bands are formed concrescence must be conceded. The formation of the vertebrate embryo may be easily regarded as a modification of the same process, and a more rational view has not yet been propounded, so far as I am able to judge. Concrescence seems, indeed, to be the very essence of Sedgwick's theory (No. 36) of the origin of the mouth and anus, and yet he rejects this view as applied to vertebrates.

It will be obvious to the reader of the foregoing pages that I regard as untenable those theories according to which the somites of segmented animals are derived from gut-pouches. It is not the archenteron, nor yet the mesenteron, in which metamerism first exhibits itself.

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EXPLANATION OF PLATE IV.

Reference Letters.

a, left macromere.
b, right macromere.
c, anterior and median macromere.
cl, cephalic lobe.
ec, ectoderm.
en, entoderm.
enp, entoplast.
ep, epidermal layer.
m, mesoderm.
m', lateral cell-row.
nb, teloblasts of the neuronephric stratum.
nc, nerve-cord, neural cell-rows, neuroblast.
nph, nephridial rows.
p, posterior end of cephalic lobe.
x, right mesoblast.
xy, left mesoblast.
y, yolk.

FIG. 1. *C. complanata* from Naples. Surface view of germ-bands in an equatorial position. The white blotches in the yolk are entoplasts (*enp.*) × 120.

FIG. 2. *C. complanata* (Naples). Transverse section near the middle of the egg, in a little earlier stage. The contrast in color between the superficial and the deeper cells of the ectoderm has been made too great by the lithographer. × 120.

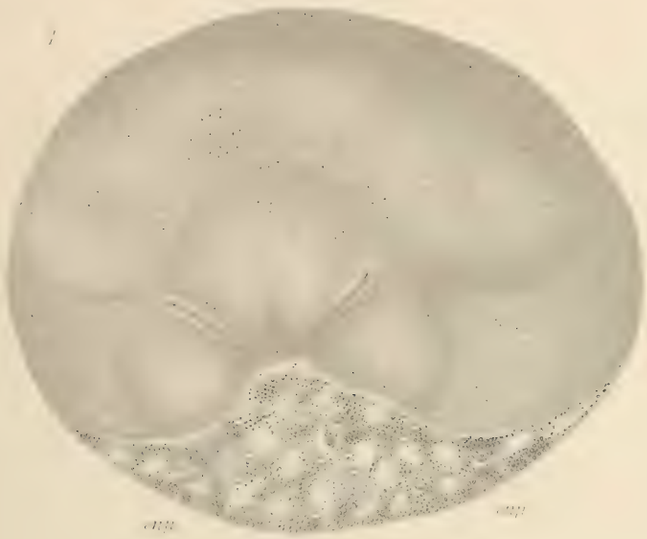
FIG. 3. *C. complanata* (Naples). Median sagittal section of the same stage. × 120.

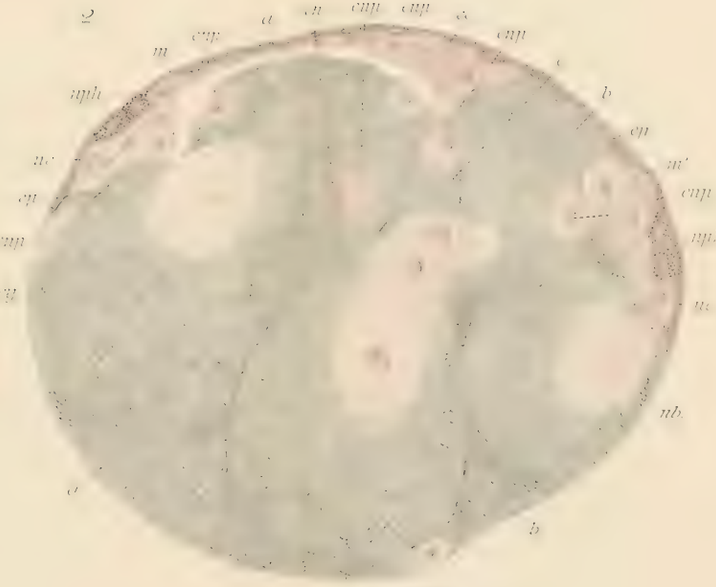
FIG. 4. Cephalic lobe and underlying yolk, from the same series of sections, but a little to one side of the median plane. × 280.

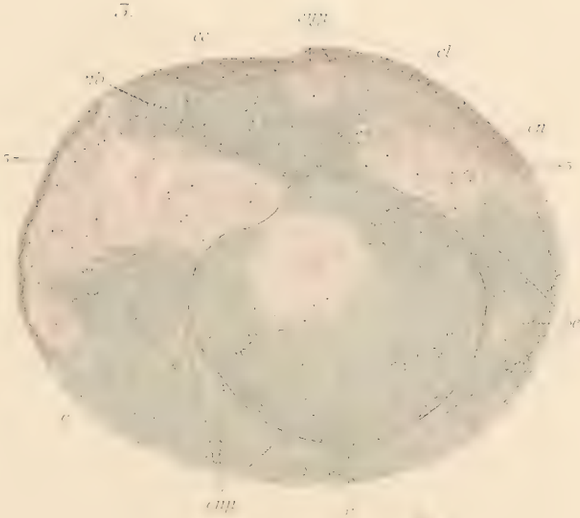
FIG. 5. An obliquely horizontal section of the same stage, at the level of the arrow 5-5 in Fig. 3. × 120.

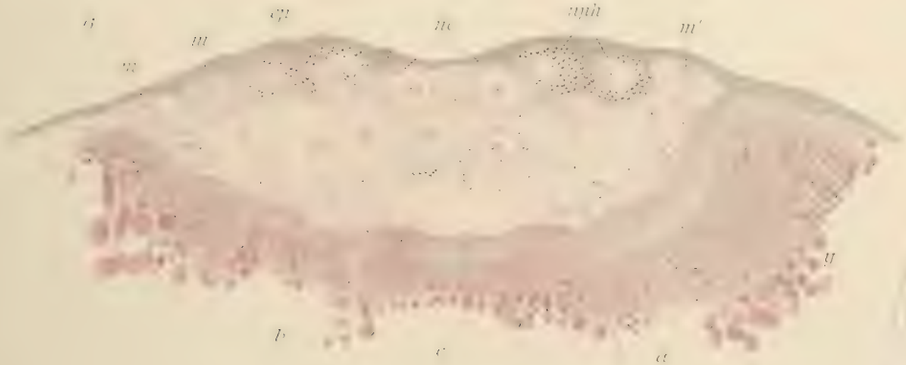
FIG. 6 *C. parasita* (Cambridge, Mass.). Transverse section of the embryo, when the germ-bands are a little more than two-thirds closed. Section is just in front of the unclosed portion of the bands. × 280.

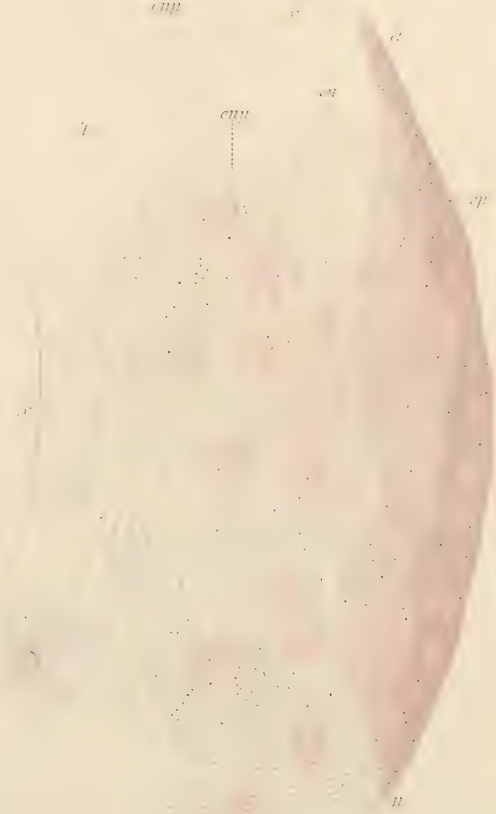
FIG. 7. From the same series, just behind the point of closure.











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EXPLANATION OF PLATE V.

Reference Letters.

c, coelom.
en, entoderm.
ep, epidermis.
g, lateral ganglia.
gl, larval glands.
lc, longitudinal commissures.
m, mesoderm.
m', lateral cell-row.
nc, nerve-cord — neural rows.
nph, nephridial rows — nephridia.
oe.c, œsophageal collar.
s, septum.
sb,^{I} anterior pair of sense-bulbs.
sb,^{II} posterior pair of sense-bulbs.
sex, sexual cells.
sp, somatopleure.
spp, splanchnopleure.
st, mouth.
st.d, stomodæum.
y, yolk.

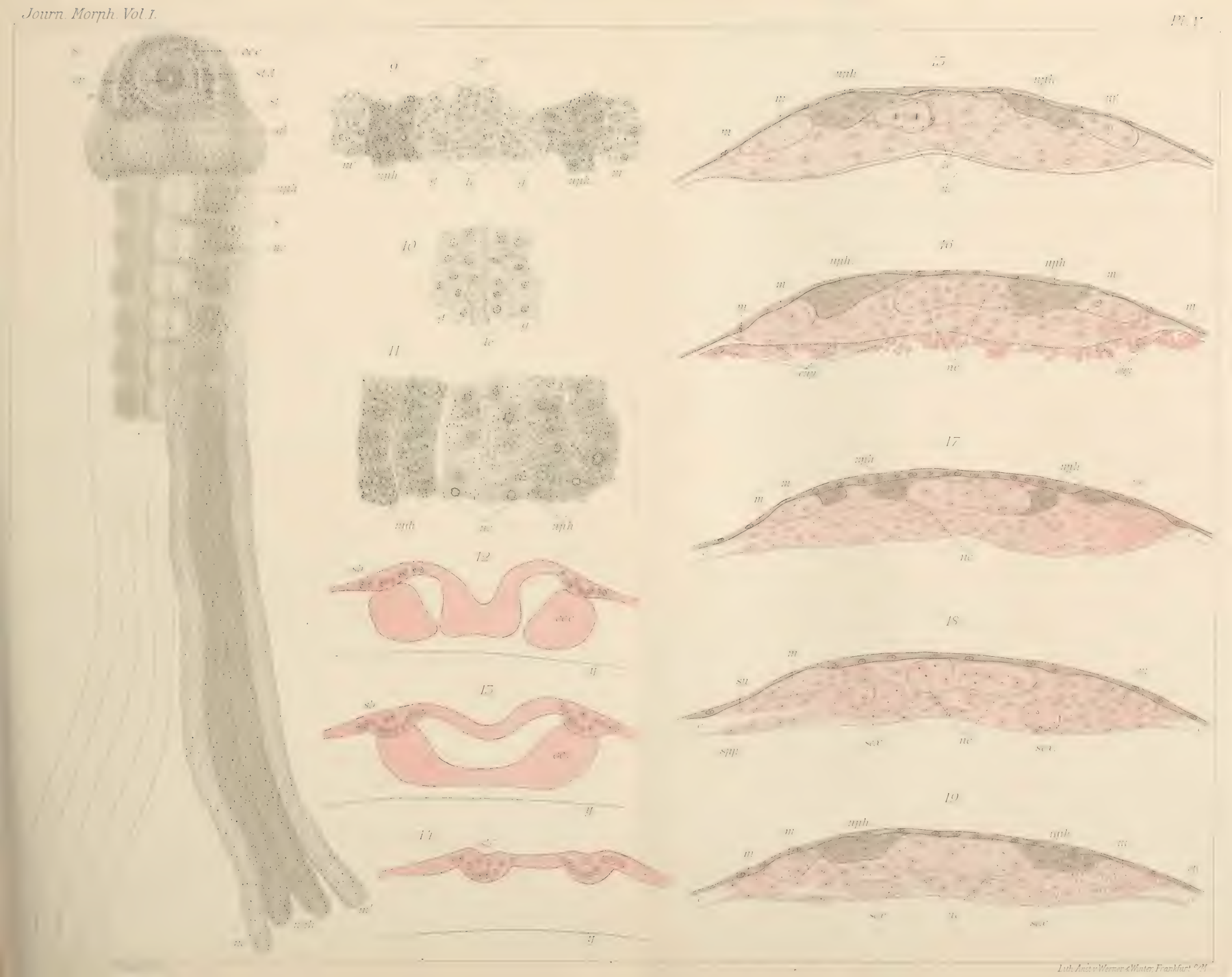
FIG. 8. *C. parasita.* Surface view of the embryo when the germ-bands are about two-thirds closed.

FIGS. 9–11. Three portions of a horizontal (frontal) section of the embryo, after the complete closure of the germ-bands, showing different stages in the development of the neuronephric stratum. Fig. 9, near the end of the first third; Fig. 10, near the beginning of the last third; and Fig. 11, very near the hind end. × 280.

FIGS. 12–14. From a series of transverse sections of the head at time of hatching. Fig. 12 hits the dorsal edge of the stomodæal ingrowth, and grazes the anterior pair of sense-bulbs (*sb*^{II}); Fig. 13 is the next section above, taking in a strip of the œsophageal collar and the centre of the sense-bulbs; Fig. 14 is the fifth section (= .0075^{mm}) above Fig. 13, and hits the posterior pair of sense-bulbs (*sb*^{II}); *y* = the line of the yolk. The position of these sections is shown by the arrows in Fig. 29. × 280.

FIGS. 15–19. Selected from a series of transverse sections through the hind end of the embryo at time of hatching, showing an early stage in the development of the nerve-chain and the nephridia. The sections run from near the middle of one somite to the middle of the next in front. Fig. 15 gives the first section; Fig. 16, the second; Fig. 17, the fourth; Fig. 18, the fifth; and Fig. 19, the seventh.

FIG. 18 is taken on the boundary line of the two somites. × 280.



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EXPLANATION OF PLATE VI.

Reference Letters.

c, cœlom.
d, ducts of larval gland-cells.
en, entoderm.
enp, entoplasts.
ep, epidermis.
g, ganglia.
gl, larval gland-cells.
m, mesoderm.
nc, nerve-cord.
nl, neurilem.
p, proboscis.
s, septa.
sb', anterior pair of sense-bulbs.
sgl, salivary glands.
sp, somatopleure.
spp, splanchnopleure.
st.d, stomodæum.
sub.œ.g, subœsophageal ganglia.
sup.œ.g, supercesophageal ganglia.
tr, transverse nerve-fibres.
y, yolk.

FIG. 20. *C. parasita.* Median sagittal section of an embryo in which the germ-bands are one-half closed. × 280.

FIG. 21. Similar section of a stage in which the germ-bands are nearly closed. × 280.

FIG. 22. Middle region of ventral side (from the same section), showing entoplasts in the periphery of the yolk. × 280.

FIG. 23. Transverse section through the region of the larval gland-cells at time of hatching. × 280.

FIG. 24. Near the middle of the ventral side. From a sagittal section following that seen in Fig. 28.

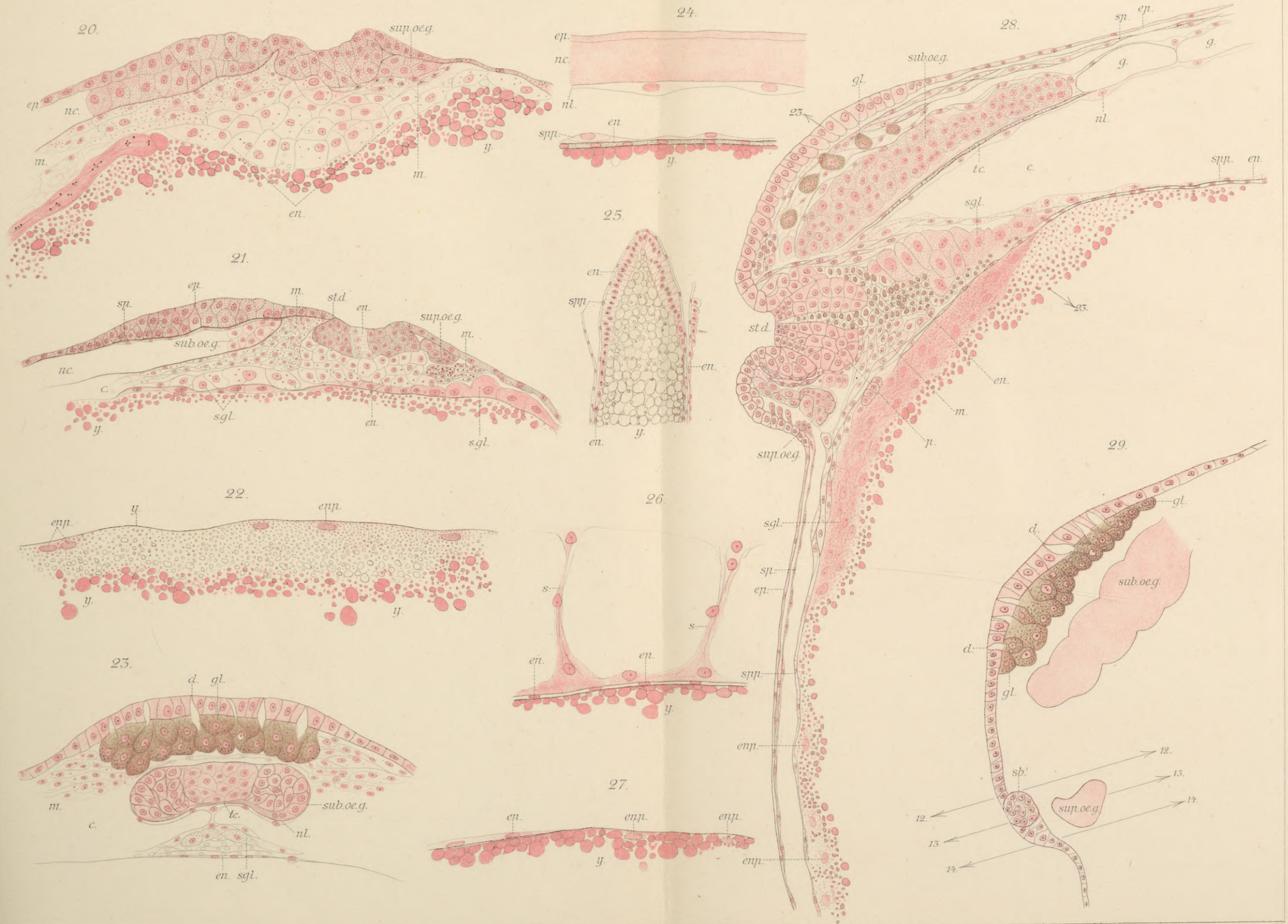
FIG. 25. *C. marginata.* Nine days old. Longitudinal section of one of the anterior gastric diverticula. The dorsal half alone is shown. × 280.

FIG. 26. *C. parasita.* Same series as Fig. 28. Middle region of ventral side, showing the septa and an early stage of the entoderm. × 465.

FIG. 27. From the same section, nearer the hind end. × 465.

FIG. 28. Median sagittal section at time of hatching. × 280.

FIG. 29. Sixth section from that shown in Fig. 28. × 280.



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